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Inhibition of the Experimental Production of Liver Cancer by Millet Feeding

By

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In the original experiment[1] to produce liver cancer by butter yellow (dimethylaminoazobenzene), the animals were fed on husked but unpolished, unwashed rice as staple food. Soon after the discovery, dietary effects on the carcinogenesis were found. While polished rice[2] augmented the production of liver cancer, bread[3] and rice germ[4] retarded it.

However, as *Chô*[5] could not alter the result by the addition of defatted yeast, the researches were directed to the fat-soluble constituents, and rice bran oil[6], rice germ oil and certain of their fractions were fouud to show the inhibitory effects.

On the other hand, *Maisin*[7] reported that the rats fed on rye flour were more resistant to the carcinogenesis by butter yellow than the animals fed on polished rice. In the present experiment, the effects of millet feeding were studied.

Experimental Methods

Out of stock albino rats of mixed strain, which were under careful observations at least for a month, 100 rats in good conditions were chosen; a half were used for millet feeding while the other half were fed on rice as controls. Husked and polished rice and only husked Japanese millet were used unwashed, and for 1 kg was mixed 20 cc of about 3 per cent

butter yellow solution in olive oil. Besides these foods, green vegetables, dried sardines and water were adequately given.

Results

The animals in the millet group showed altogether better physical condition with lower death rate (Fig. 1). In 158 days 40 rats died in the millet group instead of 45 in the rice group. 4 rats in the rice group, which died after 89 days, showed nodular hyperplasia and cirrhosis in the liver. In 2 cases the livers weighed more than the normal and hyperplasia was microscopically somewhat atypical. However 6 rats in the millet group, which died during the corresponding period, showed no significant changes in the liver, except 2 cases, in which the liver surface was only slightly uneven (Tab. 1).

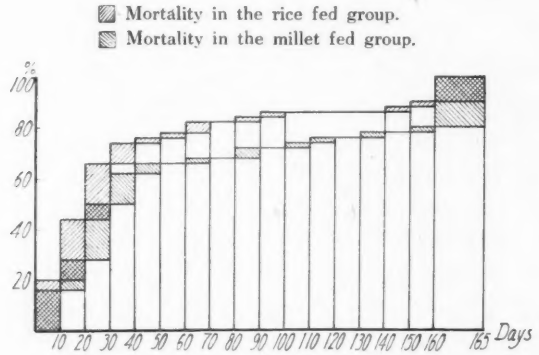

Fig. 1. Mortality for every 10 days in both groups.

On the 165th day, all the surviving animals, 5 in the rice group and 10 in the millet group, were sacrificed for internal examinations. In the rice group, all the livers showed tumour formations (Fig. 2); 4 of 5 were liver cell carcinomas (hepatomas) of the solid or trabecular type (Fig. 3), and one was atypical nodular hyperplasia (according to *Maruya*'s classification[8]) with cirrhosis. In the millet group, no liver cancer was found. The livers had practically normal appearances, only with microscopical degenerative changes in 5 of 10 rats (Fig. 4) and at most with slight cirrhosis in the other 4 rats. But in a single case, although the liver looked macroscopically normal, nodular hyperplasia of the liver cells was detected in the microscopic examination, but showing neither fibrous proliferation nor

No. 570 No. 591

Fig. 2. Two examples from the rice experiments. Development of liver cancers on the 165th day.

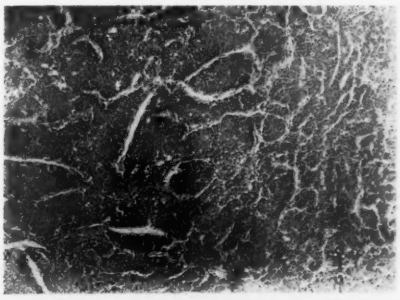

Fig. 3. Hepatoma of a trabecular type in a liver section of No. 570.

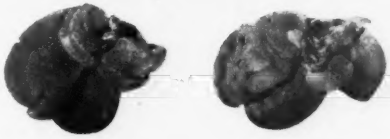

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Fig. 4. Two examples from the millet experiments. Practically normal appearances of the liver on the 165th day.

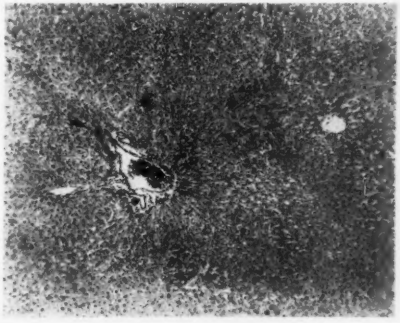

Fig. 5. Only slight degenerative changes in a liver section of No. 346.

Table 1.

	No.	Sex	Days of experiment	Body weight (g)		Liver weight (g)	Liver changes
				Initial	Final		
Rice group	596	♀	89	150	80	5.0	Nodular hyperplasia, Cirrhotic
	585	♂	92	105	110	6.9	Nodular hyperplasia, Cirrhotic
	588	♀	150	85	110	15.8	Nodular hyperplasia, Cirrhotic
	576	♀	158	110	110	15.5	Nodular hyperplasia, Cirrhotic
	576	♂	165	105	180	13.2	Hepatomatous
	563	♂	165	115	150	13.4	Hepatoma
	570	♀	165	105	160	13.6	Hepatoma
	572	♀	165	100	195	12.0	Hepatoma
	591	♂	165	110	90	14.5	Hepatoma
Millet group	308	♀	89	105	75	5.6	Normal
	313	♂	89	130	75	5.9	Slightly cirrhotic
	344	♀	106	125	75	4.1	Normal
	347	♂	115	140	70	4.5	Slightly cirrhotic
	328	♀	131	100	105	5.8	Normal
	309	♂	151	95	65	5.1	Normal
	317	♂	165	100	170	7.5	Normal
	321	♂	165	95	150	6.5	Normal
	340	♀	165	95	120	6.8	Normal
	346	♀	165	85	140	7.4	Normal
	348	♀	165	115	170	10.6	Normal
	302	♀	165	95	100	5.6	Slightly cirrhotic
	320	♀	165	120	160	8.5	Slightly cirrhotic
	327	♂	165	100	140	8.7	Slightly cirrhotic
	333	♂	165	110	160	9.8	Slightly cirrhotic
	325	♂	165	130	155	5.5	Nodular hyperplasia

proliferation of bile duct epithelium, similarly as seen in the prolonged experiment with a small dose of butter yellow[9] (Tab. 1).

Discussion

In all the cases, specimens were taken from several parts of the livers and carefully examined under the microscope. However, as described above, no liver cancer was found in the millet experiments; and the livers were practically normal or slightly degenerative and cirrhotic, and in a single

case just nodularly hyperplastic. On the contrary, nearly all the cases showed production of liver cancer in 165 days in rice experiments; and even in shorter periods nodular hyperplasia and cirrhosis were marked. It is therefore quite clear, that millet feeding could hinder the production of liver cancer by butter yellow.

Although there were distinct differences in the effects by feeding on rice and millet, the changes even in the rice group were already less significant, compared with similar control experiments, done in the same laboratory. As the supply of medical olive oil became unfavourable, industrial olive oil was used instead in the present experiments. The differences in their compositions might have caused the different effects on the carcinogenesis.

The searches for the inhibiting element or elements should be exciting. *Nakahara*[10] failed to show the inhibitory effect in vitamin B complex, such as B_1, B_2 and B_6, or in nicotinic acid. *Okamoto*[11] could not reduce the inhibitory effect of rice bran oil by destroying vitamin E in it. However, *Futagawa* and *Okamoto*[12] suggested the unsaponifiable portion of rice bran oil to be effective. *Simbo* and *Sato*[13] succeeded to inhibit the production of liver cancer by crystalline substances, separated from the unsaponifiable portion of rice germ oil. It was analytically found that 80 per cent of the crystalline substances were represented by β-sitosterol with a small amount of α_2-sitosterol and traces of dihydrositosterol, stigmasterol and mericyl-alcohol. The distributions of these substances are considered to be wide in the different plant grains and germs. They may be contained also in millet and, although less, in the industrial olive oil.

The inhibiting mechanism is of course unknown. The effective substance may hinder the absorption of butter yellow in the intestines, may show antidotal or neutralizing effects against butter yellow, or may interfere with the carcinogenesis. According to *Imai*[14], the crystalline substances mentioned above, and also β-sitosterol could decrease the rate of tissue growth in vitro by destroying the proliferating cells. This, however, does not give a solution to the above questions in any way.

However, if the incidence of spontaneous liver cancer among C_3H mice could be decreased by feeding on millet, a road to an important knowledge would be opened. It is a well known fact that in the Far East primary liver cancer is of frequent occurence. Such peculiar prevalence of liver cancer is considered to be closely connected with rice eating. In

China, though equally Chinese poeple, there is a wide difference between North China and South China in their living conditions, especially in dietary. Among the Chinese in North China, whose staple food is millet, the incidence of liver cancer is much less common than among those in South China, who live mainly on rice.

Summary

In the butter yellow experiments to produce liver cancer, millet was given to the rats as staple food instead of rice. While hepatoma was produced in a high percentage in the controls, no cancer was found in the millet experiments and the changes in the livers were not marked at all.

We wish to thank Prof. *Kinosita* for his suggestion of this study and his help in preparing the manuscript.

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要　旨

黍食に依る實驗的肝癌發生の抑制

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（昭和16年1月18日受付）

白鼠に Butter yellow を經口的に投與して肝臟癌を發生せしめんとする場合，主食餌として白米の代りに，玄米，裸麥，パンを使用し或は白米主食に胚芽，米糠油，酵母，牛肝粉末等を添加することによつて肝癌の發生は遲延し又は抑制せられることが漸次明かとなつてきた．

今回の實驗に於ては，主食餌として白米の代りに日米黍を使用し白米主食のものと對比して觀察した．兩群夫々50匹の白鼠をもつて實驗を始めたが，165日間の實驗に耐えたものは白米群では5匹，黍群では10匹であつた．而かも白米群の4匹は肝硬

變を伴ふ立派な肝癌(Hepatom)を發生し殘りの1匹は可成り著明な肝硬變を伴ふ肝細胞の結節狀增殖の像を示すものであつた．然るに黍群では10匹の中5匹は殆んど正常に近く，4匹は輕度の結締織增殖を示し他の1匹は肝臟重量も寧ろ輕く肉眼的には何等異常が認められなかつたにも拘らず組織學的に只一ケ所に結締織竝に膽管の增殖を伴はない肝細胞の結節狀增殖が認められた．

卽ち，日本黍を主食ミすることによつて Butter yellow 肝癌の發生は可成り著明に抑制せられることを明らかにすると同時に，白米を主食ミする場合に見られる肝硬變も極めて輕度であることを知つた．目下，是等發癌抑制食餌中の有效物質の分析的研究を進めてゐる．然し乍ら癌研究の窮極は自然癌發生の抑制に在るのであつて，幸ひ今日肝癌を自然に發生する血統の C_3H 株のマウスを持つてゐるので，此處に重要な進路があるわけである．

直腸癌根治手術後4年にして後腹膜に發生したる細網肉腫の一例

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（圖版 XV—XVI）

（昭和16年1月14日受付）

本症例は筆者の同鄕の先輩故文學博士喜田貞吉氏であつて，同博士は昭和10年直腸癌に罹患されたが，幸にして根治手術により起死回生の喜びを得られた．後4年再び腹部に腫瘤を生じ，遂にそのために仆れられたのであるが，自ら再び起つ能はずと覺られた時，死後剖檢によりその病變を明かにされ度く，之によつて醫學に多少なりとも貢獻し得るならば本懐であるとの意を家人に遺言された．筆者は偶々其の組織學的檢索に携はるの機會を與へられたのであるが，檢索の結果は下記の如き興味ある所見を得たので，同博士の遺志にそひ得ることを喜び，こゝに其の結果を報告し博士の靈前に捧げ度いと思ふ．

緒　言

同一患者に於て2種又はそれ以上の互に全く無關係の原發性腫瘍を見る場合，大體之を3つの場合に分つことが出來る．卽ち第1に其の何れもが良性腫瘍なる場合，第2にその何れかが良性腫瘍であり，他が惡性腫瘍なる場合，第3にはその何れもが惡性腫瘍である場合である．而してその各の場合の頻度を考へて見るに，第1の場合は日常屢々遭遇するもので珍しいものではなく，第2の場合も亦それ程稀有といふ程のものではない．之に反し第3の場合，卽ち2種或はそれ以上の原發性惡性腫瘍が同一個體に發生することは可成り稀有なものである．文獻に徵するに *Billello* 及び *Montanini* (1931) は8000例の解剖例中僅かに1例を見出したのみであるといひ，諸威癌研究會の統計によると癌剖檢例の1%，*Harwitz* は癌剖檢例の3%に過ぎないと言つて居る．*Schabad* 及び *Goriainowa* (1931) の統計によると，剖檢例6652例中腫瘍例は1457例でその中重複性惡性腫瘍（白血病を含む）は23例であつたと言ひ，*Hilgert* (1939) は惡性腫瘍1257例中28例の重複腫瘍を見たと報告して居る．以上の統計によつて見ると，何れにしても可成り稀なものであることが明かである．又是等の場合，その何れもが癌腫である場合と，その何れかが癌腫であり他は肉腫その他の非上皮性惡性腫瘍である場合との2つに大別することが出來る．その頻度は前者の方

が遙かに多いこと勿論である。

Pusateri は原發性惡性多發性腫瘍を3群に分ち，1) 同一系統の臟器に多發するもの(例，消化管)，2) 生理的關聯を有する臟器に多發するもの，(例，卵巢，子宮，乳腺等)，及び 3) 何等密接なる關聯を認めない臟器に多發するものゝ3つの場合を擧げて居るが，自分は此の第3群に相當し且最も稀であるとされて居る癌腫及び肉腫が相踵いで同一個體に發生した例に遭遇したので次に報告する次第である。

臨牀的事項

69歲 ♀ 1934年頃より痔疾の徵候があり，溫泉に赴き自ら治療に努めたが輕快しなかつた。其の後醫師を訪れ局所への注射療法を受けたが反つて增惡し，肛門部不快感，排便障碍，出血等の症狀が增して來た。1935年他の醫師により惡性腫瘍の疑を置かれ，試驗切除の結果直腸癌の診斷を下された。同年3月東大外科を訪れ，直腸癌は尙手術可能であるとの診斷を受けたが，事情により根治手術は受けず，暫くの間レ線治療を受けた。其の後6月に至り意を決して根治手術を受けることゝなり，6月19日順天堂醫院に於て入代博士執刀の下に直腸切斷による根治手術を受けた。當時の所見を簡單に記すると，全身の榮養は可成り良好で，上述の自覺症狀の外，直腸には壺腹部の下部前壁から左右にわたり直徑約4cmの圓形の癌性潰瘍があり，その潰瘍の下端は一部肛門外に現れて居た。尙肛門の周圍には輕度の發赤，腫脹があり，且硬結が認められた。手術は腰椎麻醉の下に *Kraske* 氏法に從つて施行された。周圍殊に攝護腺との癒著は手術を可成り困難なものにしたが，周圍の淋巴腺には認むべき腫脹を見出さなかつたことは，注目に値する所見であつた。手術により剔出された材料に就て見ると，直腸には壺腹部下部前壁より左右に擴る，直徑約6cmの腫瘤があり，その中央は潰瘍を形成して居る。下端の一部は肛門輪の外に迄及び，直腸後壁には一部未だ健康に見える粘膜が少許殘存して居る。潰瘍の邊緣は著しく隆起して居り，潰瘍底は凸凹不平ではあるが比較的平坦で，深い潰瘍の形成などは見られない。周圍との境は比較的明劃で，隆起した邊緣から直ちに健康に見える直腸粘膜に移行して居る。腫瘍の硬度は何處も一樣で可成り硬い。肛門周圍の皮下組織には可成りの硬結が見られる。直腸は潰瘍上端より7—8cm 距つた部に於て切斷されて居るが，此の部の直腸壁には何等病的と思はれる變化を認めなかつた。又直腸周圍にも轉移を思はせる淋巴腺腫脹は全然見出されなかつた。

組織學的所見： 腫瘍の數ヶ所から取つた切片に就ての所見は大體一樣で，何れも退形成度の比較的少い，腺樣構造の著明な圓柱上皮癌の像である。細胞は大體に於て一層に排列して，大小不規則な內腔を圍んで居る。核の配列は稍〻亂れて數列になつて居る所が多く，核分剖像も可成り多數に見られる。癌組織は潰瘍緣で比較的急に健康粘膜に移行し，又深部への浸潤も少く，多

くの部分では粘膜下組織を超えて僅かに筋層に浅い浸潤を示して居る程度である．肛門外に及んで居る潰瘍底は主として肉芽様の組織から成つて居るが，その一部には明かに癌組織を認めることが出来る．

以上の所見よりすれば，此の癌腫は比較的惡性度の少いものであることが想像出来る．既往症よりして見ると可成り發病後の經過の長いことが考へられるにも拘らず，淋巴腺轉移の見出されなかつた點と一致する所見であると思はれる．

手術後經過： 術後の經過は大體に於て順調で，2ヶ月後には全く元氣を恢復して退院する運びとなつた．退院後も異常なく，其の後全く健康を恢復して，旅行，大學に於ける講義等，病前の仕事に從事して何等の支障を認めなかつた．此の狀態が1939年春迄續き，本人は勿論周圍の者も皆その永久治癒なることを確信して居たのであるが，同年3月何等の原因なく腹痛下痢を起し，且口內炎を併發した．併し之は醫師の治療により日ならずして全快した．併しその頃より何となく氣分が勝れず，6月初旬に至り腹部に不快感を覺え，且食慾の減退が著明になつた．次で自ら上腹部に鷄卵大の腫瘤を觸知し，驚いて再び順天堂醫院を訪れた．

當時の所見としては，全身の榮養は著しく衰へて元氣なく，衰弱の強い狀態であつた．上腹部には幽門部に相當して大人手拳大の腫瘤があり，移動性は全然缺除し，手術不可能の診斷を下された．レ線檢査によると，腫瘤は胃の體部及び幽門部を強く後方より壓迫せる狀態であるが，胃粘膜自體には陰影缺損なく，恐らく腫瘤は胃自身より發生したものではなく，後腹膜に發したものであらうと考へられた．其の後旬日ならずして食物の通過障碍が著明となり，患者の切なる希望があり，又腫瘤の壓迫による胃の通過障碍に對し何等か施す術があればとの希望を以て，7月2日開腹術を行つた．開腹時所見としては，レ線像に一致して胃の後部に小兒頭大の腫瘤が存し，膵臟及び胃後壁と固く癒着して居たが，胃そのものには腫瘤形成を見なかつた．腫瘤は可成り硬く，移動性は全然缺除し，肝臟には數個の灰白色胡桃大の轉移結節が認められた．以上の所見で施すに術なく，試驗開腹術に終つたが，術後衰弱頓に加り，遂に翌7月3日鬼籍に入つた．

剖檢的所見

高度の羸瘦を示す男性屍で，皮膚に異常なく，外部より觸知し得る淋巴腺腫脹を認めず．胸部臟器に著變を認めず．腹腔には腹水なし．上腹部を見るに，胃及び十二指腸と膵臟との間に約小兒頭大稍長卵圓形，表面稍々凹凸不平，暗紫色の腫瘍が存在し，胃，十二指腸は此の腫瘤のために著しく前方に壓迫せられ，且胃後壁の大部分及び膵臟前面の大部分は腫瘤と固く癒着して居る．尙肝臟後下面の一部にも腫瘤との間に癒着が見られる．又此の主腫瘤の外に，之に接し，殊に腹部大動脈の周圍に於て，多數の指頭大乃至鳩卵大の小腫瘤結節が見出されるが，何れも上述の主腫瘤と連り，全

體ミして一つの大腫瘤塊を形成して居る．主腫瘤の割面を檢するに．極めて高度且廣範圍にわたる出血が見られ，腫瘤はその半ばは出血竈ミ化し，殊に表面に近い部に於て甚しい．又出血竈以外に於て處々に壞死竈が認められる．之等の二次的變化の强い部分の間には灰白色の腫瘍組織が殘存し，深部に至る程その量が多い．之等の組織は場所によるミ可成り結締織纖維形成が著明で，肉眼的にも束をなして走つて居る像が認められ，可成りの硬さを示して居る．

次に周圍ミの關係を見るに，胃，十二指腸ミの關係は上述の通りであるが，癒着部に於ける腫瘍組織の胃壁內への浸潤は肉眼的には極めて少く，胃粘膜はよく保たれ潰瘍形成等は見られない．膵臟ミの關係は胃に於けるよりも密接で，膵頭部の一部に於ては腫瘍組織は肉眼的にも明かに膵組織內に侵入し，腫瘤ミ膵臟ミの境は一部全く不明ミなつて居る．併し他の大部分に於ては，癒着はあるが兩者の境界は一般に明劃で，膵組織には高度の硬變像が認められるのみである．大網膜は幽門部に近く一部腫瘍ミの癒着强く，腫瘍組織の浸潤による高度の肥厚が見られたが，大彎に附着する大部分に於ては全然變化を認めなかつた．肝臟には胡桃大迄の十數個の灰白色の軟い轉移結節が見出され，割面を檢するに壞死に陷る傾向が極めて强い．小腸腸間膜淋巴腺に腫脹なく，其の他の腹腔內臟器に特記す可き變化を認め得なかつた．殊に4年前直腸癌手術を受けた部分を檢したが，人工肛門部，其の周圍，竝に之に接するS狀結腸には何等腫瘍再發ミ見る可き變化を認めず，又その近くに淋巴腺腫脹を認めなかつた．

組織學的所見： 腫瘍組織は全體ミして退形成の强い腫瘍細胞よりなり，概して細胞に富むが一部には間質結締織纖維の形成著明の部分もある．腫瘍細胞ミ間質ミの關係は極めて密接で明かな蜂窩狀の構造は何處にも見られない．腫瘍細胞は異型性，多型性極めて高度で，細胞の形狀，大きさ，核の大きさ竝に形狀，その色質量等何れも種々樣々で極めて不規則である．卽ち細胞の形は圓形，紡錘形，多角形ミ種々樣々で，紡錘形にしても極めて長いもの，極めて短いものミ何れも不揃ひで，多くは1個乃至數個の不規則な原形質突起を出して居る．大きさも不同著明で，又巨大核，多核を有する巨態細胞も出現し，種々の割合に入亂れて存在して居るのが見られる．核も圓形，卵形，紡錘形，或は不規則な多形核を示し，その大きさ，色質量等全く不定であるが，色質の少い泡狀を呈するものが可成り多く，之等のものは1個又は2個の核小體の明かに認められるものが多い．核分劑像は到る處に多數見出され，直接分裂，間接分裂，多極性分裂等何れも認められる．之等の細胞は又退行性變化を示す傾向强く，到る處に壞死，出血が見られる．間質ミの關係は上述の如く極めて密接である

が，結締織纖維形成著明の部では細胞は比較的紡錘形に近いものが多く，*van Gieson* 染色によるに膠原纖維は此の紡錘形細胞との間に直接の連りを認め得る如き像もあり，恰も纖維肉腫を見る如き感のある部分が存する。併し格子纖維染色を施して見るに之等の膠原纖維の外に腫瘍全體にわたつて格子纖維の形成が極めて著明で，細胞に富む部分に於ても亦極めて細い格子纖維網が形成されて居り，而も腫瘍細胞と格子纖維との關係を注意して見るに，纖維は一部腫瘍細胞内に終り，又細胞の原形質突起との間には明かな連りを認めることが出來る。尙 *Sudan* III による脂肪染色を施して見るに，之等の腫瘍細胞の中には小脂肪滴を有するものが可成り多數に見出される。

以上の所見よりして本腫瘍は極めて惡性度の高い腫瘍なることが知られるが，腫瘍細胞と間質との關係よりして肉腫なることは疑を入れる餘地のないものであり，且一部に於て纖維肉腫樣或は多形細胞性肉腫樣の像を見ることは上述の如くであるが，併し格子纖維染色により得られた像を見る時は，此の腫瘍は細網肉腫に屬するものであるといふことが知られる。且最近緒方教授の試みられた同腫瘍の系統的分類に從へば，同腫瘍は，1) 胚芽型，2) 網狀型，3) 内皮型，4) 組織球型，5) 造血型，6) 異型（多形細胞型）の6種に分たれるが，本例は正に異型即ち多形細胞型に屬せしむ可きものであると考へられる。

尙周圍臟器に於ける組織學的所見に就て述べるに，膵臟は肉眼的に一致して，組織學的にも一部明かに腫瘍組織の膵組織内への浸潤性發育が認められ，此の部に於ては腺組織は破壞消失し，僅かに腫瘍組織の間に處々にラ氏島の殘存を認め得るのみである。併し他の大部分，殊に尾部に於ては，癒着はあるが腫瘍との境界は明劃で，腺組織の萎縮，間質の增加等著明の硬變像を認めるのみで，腫瘍の腺組織内への浸潤は見出されない。次に腫瘍との癒着部に於ける胃壁の變化を檢するに，腫瘍組織の胃壁内への侵入は極めて輕度で，ただ一部に於て筋層の間に著明の浸潤を見るに過ぎない。胃粘膜は組織學的にも全然變化は認められない。以上膵臟，胃共に腫瘍により二次的におかされた像であり，之等の臟器に原發した腫瘍であるとは全然考へ難い。腹部大動脈周圍及び主腫瘤の周圍竝に肝臟内に見出された多數の轉移結節の組織像は，大體に於て上述の主腫瘍に似てゐるが，出血壞死の傾向が特に强く，腫瘍細胞は比較的圓形に近いものが多く，多數の巨態細胞を混じて居る。主腫瘍に比して結締織纖維の形成は遙かに弱い。

以上の所見より考ふるに，本腫瘍は4年前根治手術を受けた直腸癌とは全然別個の腫瘍であり，組織學的には細網肉腫に屬するものである。その原發部位としては，胃

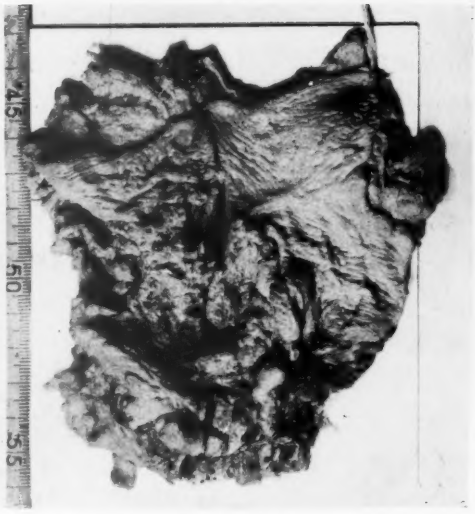

Abb. 1. Operationspräparat. Krebsgeschwür am untersten Abschnitt des Rektums.

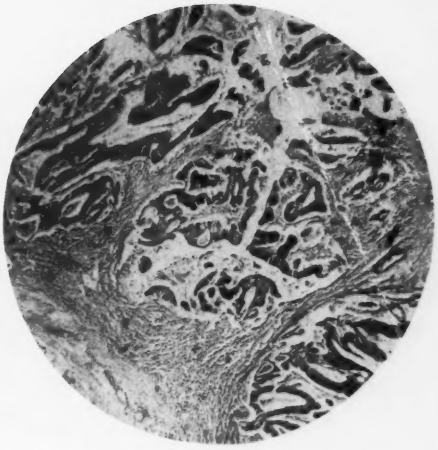

Abb. 2. Histologisches Bild des Rektumkrebses. Zylinderepithelkrebs von relativ geringer Anaplasie.

Abb. 3. Sektionsapparat. Kindeskopfgrosser Bauchtumor. Magen bedeckt die Vorderfläche des Tumors. M: Magenschleimhaut. D: Duodenumschleimhaut.

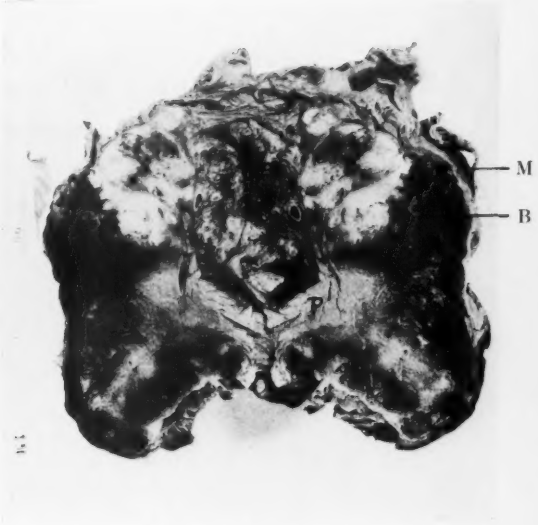

Abb. 4. Schnittfläche des Tumors. Ausgedehnte Blutungsherde. Stark gedrücktes Pankreas. M: Magen. P: Pankreas. B: Blutungsherd.

Osamu Wakabayashi: Ein Fall von Retikulosarkom im Retroperitoneum 4 Jahre nach Rektumkrebsoperation.

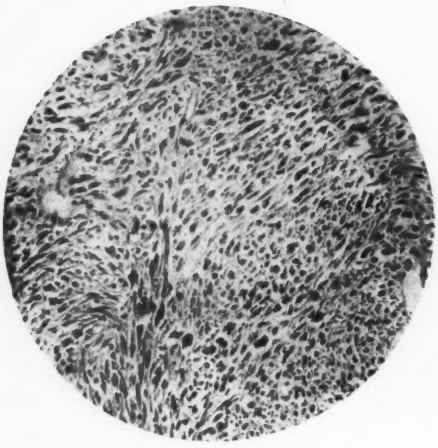

Abb. 5. Histologisches Bild des Haupttumors. Fibrosarkomähnlicher Teil des Tumors. Spindlige Zellen überwiegen.

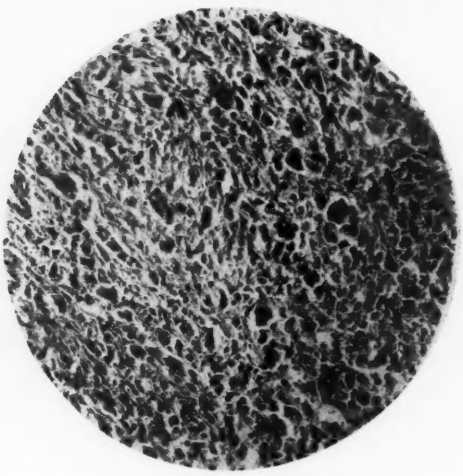

Abb. 6. Polymorphzelligem Sarkom ähnlicher Teil des Tumors. Deutliche Atypie und Polymorphie. Zahlreiche Riesenzellen.

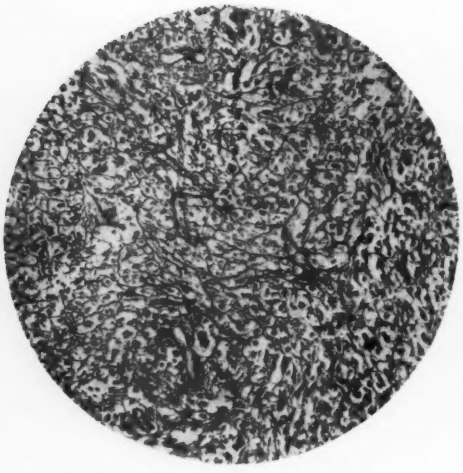

Abb. 7. Silberbild nach Bielschowski. Auffallend gute Gitterfaserbildung. Inniger Zusammenhang zwischen Gitterfasern und Geschwulstzellen. Haupttumor.

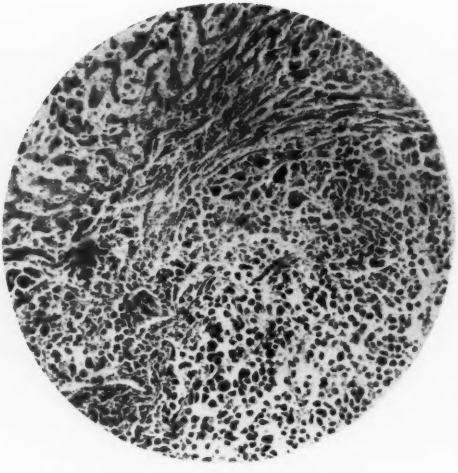

Abb. 8. Lebermetastase. Starke Atypie und Polymorphie der Geschwulstzellen. Oben atrophisches Lebergewebe sichtbar.

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ミ膵臓ミの間に位置しては居るが，其の何れの臓器に發したものでもなく，恐らくは後腹膜淋巴腺より發したものであらうミ考へられる。

直腸癌ミ細網肉腫が相踵いで同一個體を侵した稀な一例ミして報告する次第である。

稿を終るに臨み御校閲を賜つた恩師緒方教授，瀧澤助教授に滿腔の謝意を表し，且臨牀的事項の記載，組織學的檢査の材料に就きあらゆる御便宜を與へられた，順天堂醫院入代博士，柿原博士竝に佐藤勉博士の御厚意に鳴謝する次第である。

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Auszug

Ein Fall von Retikulosarkom im Retroperitoneum 4 Jahre nach Rektumkrebsoperation

Von

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(Leiter: Prof. *T. Ogata*)

(Mit TAFELN XV–XVI)

(Eingegangen am 14. Jan. 1941)

Das Vorhandensein von mehr als zwei primären bösartigen Geschwülsten bei ein und demselben Individuum ist ein äusserst seltenes Vorkommen. Ich hatte nun neuerdings die Gelegenheit einen solchen Fall histologisch zu untersuchen. Ein 69jähriger Mann verspürte im fahre 1934 ein unangenehmes Gefühl in der Analgegend und wurde auf Hämorrhoidalknoten ärztlich behandelt, aber die subjektiven Beschwerden besserten sich nicht. Im März 1935 sprach ein Arzt den Verdacht aus, dass ein bösartiger Tumor vorliegen könne, und durch den Probeausschnitt wurde die Diagnose auf Rektumkrebs bestätigt. Daraufhin besuchte der Kranke die chirurgische Abteilung der Universitätsklinik zu Tokyo Die Diagnose damals war „noch operabler Rektumkrebs" aber durch gewisse Umstände wurde er nicht sofort radikal operiert, sondern eine Zeitlang mit Röntgenstrahlen behandelt. Erst

im Juni 1935 hat er sich zur Operation entschlossen, die von Doktor *Yatusiro* in der chirurgischen Abteilung des Jyuntendospitals ansgeführt wurde. Unter Lumbalanästhesie nahm man Rektumamputation nach Kraske vor. Die Verwachsung mit der Umgebung besonders mit der Prostata machte die Operation etwas schwierig; denn nicht geringe Mühe verursachte die Ablösung, wobei das Tumorgewebe vollständig entfernt wurde. Keine nennenswerte Lymphknotenschwellung. Postoperativer Verlauf glatt und nach zwei Monaten geheilt entlassen. 4 Jahre lang danach war er vollarbeitsfähig ohne Beschwerden. Im Frühling 1939 verspürte er wieder ein unangenehmes Gefühl im Bauch, begleitet von Diarrhoe und akuter Stomatitis. Trotzdem beiderlei Beschwerden durch Behandlung bald geheilt waren, schien doch das Allgemeinbefinden seit damals nicht ganz dasselbe. Im Juni bemerkte er zufällig einen hühnereigrossen derben Tumor im Oberbauch und besuchte abermals das Jyuntendospital. Die klinische und röntgenologische Untersuchung hat dabei einen hühnereigrossen ganz unverschieblichen Tumor hinter dem Magen nachgewiesen. Probelaparotomie. Tod am folgenden Tage.

Sektionsbefund: Ein kindeskopfgrosser dunkelrötlicher Tumor zwischen Magen und Pankreas, welcher mit diesen Organen fest verwachsen war. Mehrere grauweissliche walnussgrosse Metastasen in der Leber und mehrere Lymphknotenmetastasen im Retroperitoneum. Magen und Duodenum sind vom Tumor nur gedrückt und ihre Schleimhaut gut erhalten. Das Pankreas ist grösstenteils zirrhotisch verändert und nur im mittleren Abschnitte teilweise vom Tumorgewebe infiltriert. Im alten Operationsfeld des Rektumkrebses, d. h. die Gegend des Kunstafters, der zuführenden Sigmaschlinge und deren Umgebung findet man weder Tumor, noch Lymphknotenschwellung.

Das histologische Bild des vor 4 Jahren operierten Rektumkrebses zeigte typischen Zylinderepithelkrebs von relativ geringgradiger Anaplasie, aber das des diesmaligen Tumors ist ein Retikulosarkom. Die Geschwulstzellen zeigen starke Atypie und Polymorphie. Der Tumor ist im Hauptteil einem polymorphzelligen Sarkom ähnlich teils aber auch wieder einen Fibrosarkom ähnlich, und zwar sieht man nach Silberimprägnation nach Bielschowski überall ausgeprägte reichliche Gitterfaserbildung und einen sehr innigen Zusammenhang zwischen Fasern und Tumorzellen. Nach diesem Gewebsbild gehört dieser Tumor zum Retikulosarkom und zwar, nach der neueren Einteilung von Prof. *T. Ogata*, zu dem atypischen bzw.

polymorphzelligen Typus der Retikulosarkome. Die Unabhängigkeit beider Geschwülste von einander, Zylinderepithelkrebs und Retikulosarkom, unterliegt keinem Zweifel. Wegen der Seltenheit habe ich den klinischen und pathologisch anatomischen Befund kurzfassend erörtert.

Ein Fall von papillärem Adenokarzinom des Hodens

Von

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(Vorstand: Prof. Dr. *R Kawamura*)

(Mit TAFELN XVII–XVIII)

(Eingegangen am 21. Januar 1941)

Das primäre Hodenadenokarzinom ist unter den Hodengeschwülsten selten; es kommt im allgemeinen bei Kleinkindern häufig und bei Erwachsenen wenig zur Beobachtung. In Japan sind in der mir zugänglichen Literatur nur 5 Fälle dieser Geschwulst bei Erwachsenen erwähnt worden. So berichtete *Sakaguchi* (1915) über ein vom Corpus Highmori entstandenes papilläres Adenokarzinom bei einem 70 Jährigen, *Nagamatsu* (1931) über ein Adenokarzinom eines 25 Jährigen, *Takami* (1932) über ein Adenokarzinom bei einem 37 Jährigen, *Iwakiri* (1934) über ein Adenokarzinom eines 49 Jährigen und *Chin* (1937) fand unter 15 Fällen von Adenokarzinom, deren grössere Zahl die Kleinkinder einnahmen, nur in einem Erwachsenen diese Geschwulst.

Ich erhielt zufällig das chirurgisch entfernte Material eines als primär entstandenen anzunehmenden Adenokarzinoms im rechten Hoden eines Erwachsenen (*Maeda*'s Chirur. Abt., Ambulant No. 315) und will deshalb hier über die Befunde an diesem Material berichten.

Eigener Fall

Es handelt sich um einen 38 jährigen Patienten (Türken), dessen Anamnese, ausser Erkrankung an Malaria, nichts Besonderes ergibt. Auch ist die Familiengeschichte O. B.

Vor einem $\frac{1}{2}$ Jahre bemerkte er in der rechten Hodengegend einen leichten Schmerz und dann allmähliche Schwellung.

Im Oktober 1939 Aufnahme in die Klinik. In der rechten Hodenpartie wurde ein taubeneingrosser, elastischer derber Tumor palpiert. Die anderen Organe waren ohne irgend eine auffällige Veränderung, auch liess der Harnbefund nichts Besonderes nachweisen.

Rechtsseitige Kastration wurde durchgeführt. Der Patient befindet sich etwa 1 Jahr nach der Operation bei bester Gesundheit.

Makroskopischer Befund der Geschwulst

Der taubeneingrosse Tumor, dessen oberer Teil sich von der Nähe des Caput epididymidis des Nebenhodens zum Hodenmediastinum erstreckt, ist etwa rundlich und derb konsistiert.

In der Schnittfläche ist die Tunica albuginea etwas verdickt, das normale Hodengewebe infolge des im Zentrum ausgebreiteten Tumorgewebes nach oben und unten gedrückt. Das Tumorgewebe bildet mit dem normalen Hodengewebe eine scharfe Grenze; das Zentrum des Tumors zeigt gelb grauweissliche, homogene, haselnussgrosse Flecke, wie auch das der Nekrose anheimgefallene Bild, in welchem auch braune blutige Flecke vermischt vorhanden sind. Der Nebenhoden ist im allgemeinen etwas atrophisch, sonst sind keine auffälligen Veränderungen zu bemerken. Der Samenleiter ist etwas verdickt.

Histologischer Befund

Die Tunica albuginea des rechten Hodens ist im allgemeinen verdickt und etwas bindegewebig gewuchert. Die elastischen Fasern weisen eine deutliche Zunahme auf, die Gefässzone der Substantia albuginea ist auffällig und die Hyperämie ausgebreitet. In einem Teil der Subalbuginea bemerkt man druckatrophische enge Hodenkanälchen. In der Elasticafärbung zeigt die verdickte Tunica dicke elastische Fasermembran. Die Zwischenzellen sind noch mittelmässig. Im anderen Teil ist die Tunica vom Tumor angegriffen, dazwischen bemerkt man hypertrophische Kanälchen. Ausserdem ist das Hodengewebe an der oberen, unteren und inneren Seite des Tumors weggedrängt, ist aber vom Bindegewebe mit hypertrophierten Kanälchen umgeben; in dem Hodengewebe der Umgebung lässt sich jedoch nichts Besonderes erkennen.

Der Tumor besteht aus gefässreichen, hypertrophischen Kanälchen mit Bindegewebskapsel, ist gegen das Hodengewebe scharf begrenzt und wird mit strangartigem, bindegewebigem Interstitium in zahlreiche Alveolen geteilt. Das Zentrum des Tumors selbst ist in ausgedehntem Masse von Nekrose befallen, das Parenchym solid und von grossen polymorphen protoplasmaarmen Zellen angefüllt. Der grössere Anteil zeigt aber ein adenomatös oder adenopapillomatöses Bild. Auch in diesen einzelnen Alveolen

ist homogen nekrotisierende Masse enthalten, und die Geschwulstzellen in diesem Teil sind zylindrisch oder polyedrisch, ein- oder doppelschichtig, doch die letzteren sind in grösserer Zahl. Die Grösse ist etwas ungleich, der Kern chromatinarm und hell, der retikuläre Bau deutlich; das Protoplasma zeigt eine hellrote Färbung. Mässige Karyomitose ist in den Geschwulstzellen bemerkbar.

Das Interstitium ist fibrös sehr verdickt, die Infiltration der Rundzellen sehr deutlich und in den Alveolen peripherwärts ringförmig vorhanden, die Zellen bestehen aus Lymphozyten aber auch zahlreiche Plasmazellen sind zu sehen. Die Veränderung der Gefässwand im Interstitium zeigt in einigen Arterien leichtgradige Intimaverdickung.

In Elasticafärbung haben in dem Geschwulststroma die elastischen Fasern zugenommen, aber auch in den gedrückten atrophischen Kanälchen der Umgebung des Tumors wird eine etwaige Zunahme dieser Fasern bemerkt.

Mit der Retikulumfärbung tritt in dem Alveoleninterstitium eine mehr oder weniger stärkere Zunahme auf, aber diese feinen Fasern dringen nicht in das Geschwulstgewebe ein.

In der Fettfärbung enthalten die Geschwulstzellen im allgemeinen keine Fettgranula. Die Verfettung ist bei dem der Nekrose anheimgefallenen Geschwulstgewebe deutlich. Hier ist in der nekrotisierenden Masse die deutliche Fettablagerung und im Interstitium die Fettablagerung zwischen den Fasern und die Anhäufung von pseudoxanthomatösen Zellen zu bemerken. In den atrophierten Kanälchen macht sich sogar eine hochgradige Fettablagerung bemerkbar. Die ductuli efferentes sind leicht atrophiert, aber in den Epithelzellen ist das doppelbrechende Fett merklich abgelagert.

In der Nähe der Kapsel sieht man verkalkte Corpora amylacea, die Kanälchen im gesunden Hodengewebe sind ohne Besonderheit und Spermien in reichlicher Zahl werden bemerkt. Von dem Geschwulstteil sich zur anderseitigen Tunica albuginea erstreckend bemerkt man die keilförmige Hypertrophie des Hodengewebes. Im oberen Teil der Geschwulst findet sich ein, oder in diesem Hodengewebe kleine metastatische Knoten.

(Die Glykogenreaktion erwies sich bezüglich des Fixierungsmittels unmöglich).

Nebenhodenkopf, Körper und Schwanz und Samenleiter liessen keine auffallenden Veränderungen erkennen.

Zusammenfassung

Dieser Tumor liegt zwischen dem Nebenhoden und Hodengewebe, der klinisch mit der Nebenhodengeschwulst betrachtet wurde; wie sich aber aus der genauen Untersuchung ergab, hat der Nebenhoden keinerlei Beziehung zu dieser, denn er ist im Hodenparenchym, nahe der Tunica albuginea vorhanden und liegt regionär im Corpus Highmori. Der Tumor ist taubeneigross und mit dem umgebenden Hodengewebe relativ scharf begrenzt, übt aber auf dieses einen Druck aus und schädigt somit die Tunica albuginea. Nach den histologischen Befunden weist der Tumor einen papillären Bau auf, dessen Geschwulstzellen mittelmässige Karyomitose und dessen Zentrum das Bild von Krebs mit deutlicher Neigung zur Nekrose und Blutung zeigen.

Gordon, Bell haben diese Art von Tumor in 1) einfaches papilläres Fibroadenom, 2) Adenokarzinom mit heterologer Einlagerung, 3) reines Adenokarzinom, 4) rundzelliges cirrhöses Karzinom eingeteilt. *Gioya* hat der obigen Einteilung in Bezug auf 1-3 zugestimmt, *Oberndorfer* unterschied zwei Arten, erstens eine kleinzylindrische papillär-adenomatöse Form und zweitens eine Form, die mit der von *Gioya* beschriebenen übereinstimmt.

Chin teilte wiederum diese Art in 1) typischen, zystischen und papillären Krebs, 2) atypischen Bau von Krebs und 3) spezifischen Bau von Krebs zeigende Formen ein.

Mein Fall stimmt mit der I. Form von Oberndorfer überein.

Über die Entstehungsursache dieser Geschwulst nimmt *Gioya* an, dass sie vom Epithel der Tubuli recti oder Rete testis ausgeht und dem Carcinoma Wolffianum zugehörig ist, aber *Oberndorfer* wies darauf hin, dass sie von dem Epithel der feinen Kanälchen ausgehenden Seminom sich schwer unterscheiden lässt. Meinen Befunden nach zeigte der exstirpierte Tumor noch nicht die Wucherung, von dessen Entstehungsstelle bis zu einem bestimmten Masse sich der Primärkrebs vermuten lässt. Da sich bei meinem Fall das histologische Bild des Corpus Highmori schwer auffinden liess und die Geschwulst mit diesem Teil ganz übereinstimmte, könnte man annehmen, dass sie von dem Epithel der Rete testis oder Tubuli recti in diesem Teile ihren Ausgang genommen hat.

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要　旨

睾丸乳嘴樣腺癌腫の一例

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睾丸原發性腺癌は睾丸腫瘍中稀に遭遇するものにして一般に小兒に多く成人には少し，本邦に於ける本腫瘍の成人例報告は余の渉獵せる文獻範圍に於ては坂口(1915)，永松(1931)，高見(1932)，岩切(1934)，陳(1937)等を見るのみ。余偶々成人の右側睾丸に原發したる腺癌腫の外科的材料に就き檢索したるを以て茲に報告せんとす。

患者は38歲，男子(土耳古)，Malaria に罹患せし以外特記すべき既往症なし，本症は約半年前より右側睾丸部に輕度の疼痛と腫脹を以て始まる。臨牀的には右側副睾丸部に鳩卵大の elastisch derb の Tumor を觸診す，其の他各臟器には著變を認めず。右側の Kastration を行へり。尙患者は剔出術後一ケ年を經過せるも極めて健在なり。

腫瘍の肉眼的所見. 腫瘍は鳩卵大にして上部は副睾丸の頭部附近より Hodenmediastinum を中心とする稍々圓形なるものにして灰白色を呈し，硬度睾，境界比較的明瞭。副睾丸は一般に萎縮の外著變なし，Samenleiter は稍々肥厚す。

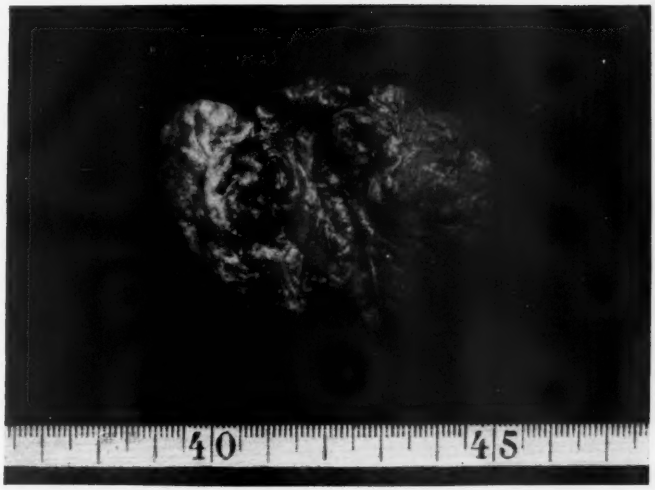

Abb. 1. Operativ hera sgenommer Tumor.

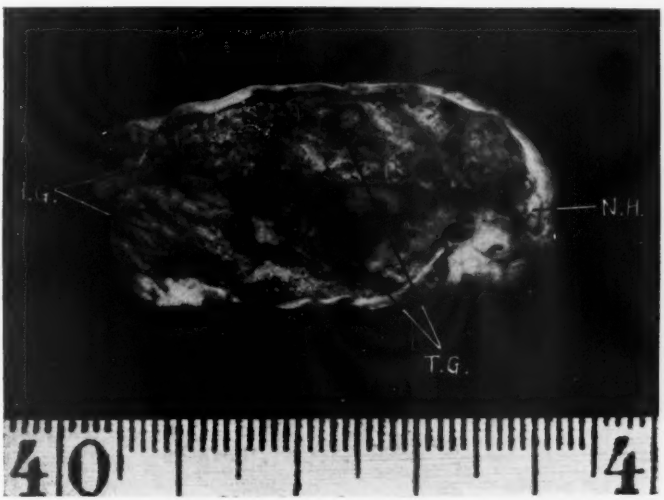

Abb. 2. Schnittfläche des Tumors.

T.G. Tumorgewebe.
H.G. atrophisches Hodengewebe.
N.H. Nebenhodenkopf.

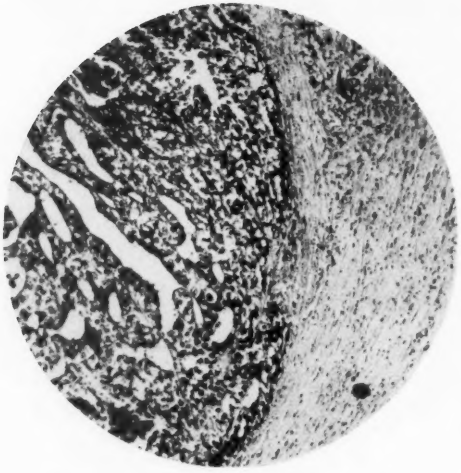

Abb. 3. Papillärer Bau des Tumorgewebe.

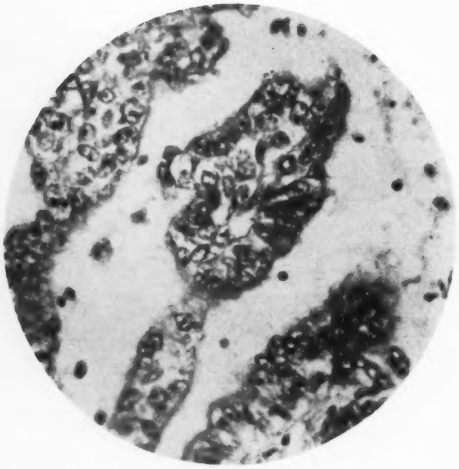

Abb. 4. Tumorzellen (in starker Vergösserung).

Abb. 5. Gitterfasern im Tumorgewebe (Silberimprägnation nach Bielschowsky).

腫瘍の組織學的所見，右側睾丸固有白膜は一般に肥厚し稍〻結締織の增殖を認め，彈力纖維の增殖著明なり，又白膜下の血管層は著明に擴張充血す，間細胞は尙中等度に認む。Tumor は gefässreich の萎縮せる細精管よりなる Hodengewebe ミ Bindegewebskapsel を以て境界され，束狀に走れる結締織性間質を以て多數の蜂巢に分たる。Tumor の中心部は ausgedehnt に Nekrose に陷り腫瘍組織は一部大なる多角狀の原形質に乏しき細胞，比較的密に配列するも，その大部分に於ては腺管狀或は腺管乳嘴樣配列をなす，卽ち該細胞は zylindrisch oder polyedrisch にして，大きさ不同なり，核は Chromatin に乏しく淡明，細網構造比較的明瞭なり，而して中等度の核分割像を認む，格子纖維染色により明かに細胞間內に於ける該纖維の增殖侵入を認めざりき．間質は fibrös に肥厚し圓形細胞の浸潤著明なり。脂肪染色標本に於ては腫瘍細胞は一般に脂肪顆粒を含有せず，然れごも前記の Nekrosenherd に於ては著明なる Verfettung を認む。

本腫瘍は以上の組織學的所見に依り明かに乳嘴樣腺狀癌にして其の發生部位は Corpus Highmori の部に一致し，尙該部に正常なる Rete testis を認めず，依つて本腫瘍は Rete testis 或は Tubuli recti の上皮より原發せるものミ思考し得べし．

擱筆にあたり御懇篤なる御指導ミ御校閲ミを賜はりし恩師川村敎授に深謝す．

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Effect of Animal Tissue Feeding on Experimental Production of Liver Cancer, Especially the Inhibiting Effect of Kidney Feeding*

By

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(With PLATES XIX–XXI)

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Introduction

The experimental production of liver cancer by feeding with an azo compound was first reported by *Yoshida*[1] in 1932, and a monographic account of the work was later published by *Sasaki* and *Yoshida*[2]. The particular azo compound used was o-aminoazotoluol, feeding of which produced liver cancer in the course of some 300 days. Although this work opened up an immense field for further investigations, it was not until the discovery by *Kinosita*[3] of another and far more powerful carcinogenic azo compound, namely, dimethylaminoazobenzol (butter yellow), that any active investigation really became possible. With proper use of dimethylaminoazobenzol, the time required for the production of liver cancer can be cut down to some 150 days, with all the incidental advantage to experimentation of this nature.

Among the numerous investigations arising as sequel to the discovery of *Kinosita*'s butter yellow liver cancer, perhaps the most significant was the series of experiments on the dietary effect on the production of liver cancer.

Recent experiments[4~6] in this laboratory demonstrated that liver feeding very strikingly inhibited the production of liver cancer due to the administration with food of dimethylaminoazobenzol. Previously several reports had appeared indicating that the diets may have some effect on the experimental liver cancer production, but, so far as we are aware, no

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such drastic inhibition as effected by liver feeding has been brought about by any other dietary means. The inhibiting effect of liver feeding was of such an extent as to prevent the development even of cirrhosis, which is the usual forerunner of liver cancer, and the liver remained macroscopically normal for 150~162 days in all and for 200 days in most of the cases. Without liver feeding, liver cancer developed in 50~100 per cent of the rats in 150~162 days.

Pending the elucidation of the nature of the liver constituent which is responsible for this marked inhibiting effect, it was considered to be of interest to try the feeding with animal tissues other than liver, and for this purpose we carried out experiments with beef kidney, spleen, muscle, brain, lung, fore and glandular stomachs, small intestine, pancreas and testicle, studying at the same time the effect of bile feeding. The results showed that kidney feeding alone of all these trials brought about a definite inhibition of liver cancer production, although the degree of this inhibition was not as high as that attained by liver feeding. The records of all these experiments will be given in the following lines.

Experimental Methods

Experimental procedures adopted in all the experiments described in this paper were entirely the same as those of our previous experiments on liver feeding so as to permit a ready comparison of the results. Briefly, these procedures were as follows: —

Each experiment was started with 40~50 normal albino rats, which were fed *ad libitum* on the mixture of polished rice (880 g), dried tissue powder (100 g) and olive oil solution of dimethylaminoazobenzol (20 g). In the case of bile feeding the proportion to polished rice was modified as will be detailed later. In all the cases, a small slice of fresh carrot was given to each rat every other day as dietary supplement.

All the tissue powder was prepared by drying fresh beef tissue, which was sent through a meat chopper, in an open dish over a steam bath, and by pulverizing, exactly in the same manner as we prepared liver powder used in our previous experiments.

The amount of dimethylaminoazobenzol used was 0.2 g per 1 kg of total food mixture at the beginning of the experiment and was gradually increased to 0.6 g per 1 kg. The approximate amount of dimethylaminoazobenzol ingested by each rat was calculated from the total amount of the food

consumed.

The rats dying early in the course of experiments were discarded, and, beginning with the first animal to die with macroscopically recognizable liver change the records were made of all the rats as they died. Experiments were terminated on the 150th day by killing all the surviving rats and performing autopsy.

In expressing the nature of the liver findings the use was made of double plus (++), plus (+) plus-minus (±) and minus (−) signs indicating respectively liver cancer, cirrhotic change only, slightly uneven surface without extensive proliferation of connective tissue, and microscopically normal. In all the cases, these diagnoses were confirmed by microscopical examination. The histological nature of the liver changes macroscopically noted and variously designated as above need not now be gone into, as in making these designations we closely followed the standard and criteria adopted in our previous investigations and fully described in one of our former publications. And there was no metastasis in any of rats examined.

Effect of Kidney Feeding

The experiments on the effect of kidney feeding will be considered first, since they afforded the only positive results in the present series of investigations.

The experiment was started with 40 rats, of which number one-half died before the 121st day without showing any liver change. On this date the first rat died with liver showing slightly uneven surface (±). Two other rats dying on the 126th and 137th days respectively were free from macroscopic liver lesion. 17 rats survived 150 days, and there were only 1 case of liver cancer and 2 cases of cirrhosis among them. Tables I and II summarize the entire results.

Table I. Experiment 1. Effect of Kidney Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♂	121	100	100	70	196	±
2	♂	126	110	115	80	304	−
3	♀	137	100	110	95	324	−

Table II. Experiment 1. (150 Days) (See Plate XIX) Effect of Kidney Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	90	115	100	340	−
2	♂	90	95	85	344	−
3	♀	90	115	110	348	++
4	♀	120	125	95	368	−
5	♀	110	130	110	392	−
6	♀	95	120	115	392	±
7	♂	100	115	110	400	±
8	♀	100	120	120	404	−
9	♀	100	120	120	436	±
10	♀	110	125	110	440	±
11	♀	100	130	130	440	+
12	♀	110	125	110	448	+
13	♀	100	120	120	452	±
14	♀	110	150	150	468	±
15	♂	100	150	145	492	−
16	♀	100	125	95	504	±
17	♂	95	150	150	512	−

−: 7(41.1%) ±: 7(41.1%) +: 2(11.8%) ++: 1(5.9%)

Table III. Experiment 2. (150 Days) (See Plate XX) Effect of Kidney Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	75	140	120	288	−
2	♂	100	135	130	304	−
3	♀	80	125	115	316	−
4	♂	150	135	135	324	−
5	♀	80	135	130	326	±
6	♂	150	170	150	344	+
7	♀	120	150	120	348	−
8	♀	110	150	130	352	−
9	♀	100	150	145	356	−
10	♀	90	130	120	360	−
11	♂	160	165	165	360	−
12	♂	90	130	120	364	−

13	♂	125	170	160	368	−
14	♂	130	170	150	396	−
15	♂	170	190	180	400	−
16	♂	110	135	130	404	−
17	♂	120	145	125	408	−
18	♂	130	170	145	412	−
19	♂	210	215	150	426	±
20	♂	220	220	190	452	−

−: 17(85.0%) ±: 2(10.0%) +: 1(5.0%)

Table IV. Experiment 3. (150 Days) (See Plate XXI) Effect of Kidney Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	70	100	85	228	−
2	♀	80	115	110	256	−
3	♀	60	105	90	280	−
4	♀	105	140	110	288	−
5	♂	70	125	115	292	−
6	♀	90	145	130	292	−
7	♀	80	135	115	292	−
8	♀	65	125	100	304	−
9	♂	80	110	90	308	−
10	♀	100	130	115	308	−
11	♂	70	125	115	316	−
12	♀	100	145	125	320	−
13	♀	100	125	115	328	−
14	♀	75	110	105	332	−
15	♂	60	140	130	332	−
16	♀	70	130	120	336	−
17	♂	70	110	100	336	−
18	♂	110	150	125	336	−
19	♂	150	160	145	340	−
20	♂	100	120	120	344	−
21	♂	80	120	110	344	±
22	♂	170	190	160	388	−
23	♂	130	150	120	400	−
24	♀	90	140	130	462	−

−: 23(95.8%) ±: 1(4.2%)

The above experiment was repeated two more times, each time with a set of 40 rats, and the data are shown in Tables III and IV. As is shown in the last column of each table, no liver cancer is found, the only liver changes being 1 case of cirrhosis (+) and 2 cases of liver with slightly uneven surface (±) in the Table III, and only 1 case of liver with slightly uneven surface (±) in the Table IV. These results confirmed that of the first experiment, and demonstrated that kidney feeding definitely inhibited the production of liver cancer. However, the degree of this inhibition was not as striking as in the case of liver feeding.

Liver Cancer Production on Polished Rice Only

The ready development of liver cancer on the usual laboratory diet is so fully demonstrated by *Kinosita*[3], that control experiment may be superfluous. Nevertheless, for the sake of direct comparison the results of experiments with polished rice diet may be quoted.

In these experiments all the surviving rats were killed on the 150th day, and the liver findings and other data are tabulated in Tables V and VI. The striking inhibition of liver cancer production due to kidney feeding may be obvious from these data.

Table V. Experiment 4. (150 Days) Polished Rice Alone.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♂	45	70	70	292	+
2	♀	65	85	80	300	++
3	♀	45	85	85	302	++
4	♂	50	75	75	305	+
5	♂	60	85	85	310	++
6	♀	50	80	80	330	++
7	♂	60	85	85	343	+
8	♀	70	100	100	350	+
9	♂	50	90	90	354	+
10	♀	85	90	90	370	+
11	♂	55	110	110	371	+
12	♀	70	90	90	381	++
13	♀	70	95	95	394	++
14	♀	70	115	110	408	++
15	♂	55	100	90	410	++

16	♀	100	105	80	412	+
17	♂	60	110	110	420	+
18	♂	55	90	90	456	++
19	♀	55	110	110	460	++
20	♂	60	115	115	476	++
21	♂	60	120	120	477	+
22	♂	70	110	110	478	+

−: 0(0.0%) ±: 0(0.0%) +: 11(50.0%) ++: 11(50.0%)

Table VI. Experiment 5. (150 Days) Polished Rice Alone.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	90	90	90	298	++
2	♀	80	90	80	307	++
3	♀	110	110	90	315	++
4	♀	100	110	110	326	++
5	♀	90	100	100	331	++
6	♀	115	120	110	333	++
7	♀	100	100	90	334	++
8	♀	95	100	90	341	++
9	♀	90	100	100	342	+
10	♀	90	100	90	348	+
11	♀	120	120	110	354	+
12	♀	120	120	90	361	++
13	♀	105	105	80	362	++
14	♀	65	100	100	366	+
15	♀	100	100	100	372	++
16	♀	90	100	80	383	++
17	♂	100	110	110	383	++
18	♀	105	105	100	385	+
19	♀	110	115	100	389	++
20	♀	115	115	100	390	+
21	♂	120	120	100	403	+
22	♂	105	110	110	407	+
23	♀	110	115	100	413	++
24	♂	115	120	120	436	+
25	♀	100	110	110	436	++
26	♀	115	120	100	446	++
27	♀	120	135	130	449	++
28	♂	110	125	100	460	±
29	♀	130	130	110	606	+

−: 0(0.0%) ±: 1(3.4%) +: 10(34.5%) ++: 18(62.0%)

Effect of Spleen Feeding

Of 40 rats with which the experiment was started as many as 28 died early in the progress of the experiment, without showing any relevant change in the liver. On the 55th day, one rat died with liver showing slightly uneven surface (±), and from this time on six other rats died with more or less cirrhotic livers, as is shown in Table VII. On the 150th day, there were only 5 surviving rats. Two of them showed liver cancers. Table VIII

Table VII. Experiment 4. Effect of Spleen Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♂	55	175	175	100	112	±
2	♂	58	160	160	110	152	±
3	♂	71	170	170	90	222	+
4	♀	72	130	130	60	158	+
5	♀	82	160	160	70	188	+
6	♀	119	130	130	75	405	+
7	♂	144	190	190	150	498	±

Table VIII. Experiment 4. (150 Days) Effect of Spleen Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♂	160	160	125	540	++
2	♂	180	180	125	576	±
3	♂	190	190	110	603	++
4	♂	180	180	135	616	+
5	♂	190	190	170	640	+

−: 0(0.0%) ±: 1(20.0%) +: 2(40.0%) ++: 2(40.0%)

brings together the individual records of all these rats. As may be seen from the table, the addition of the spleen did not produce any perceptible effect on the production of liver cancer.

Effect of Muscle Feeding

The muscle used was lean beef. Experiment was started with 40 rats, 22 of which died too early to be taken into account. The individual records

of all the remaining rats are tabulated in Tables IX and X. There was no inhibition of the production of liver cancer.

On 75th day, one rat died with cirrhotic liver (+), and from this time on, four other rats died with recognizable changes in the liver. 13 rats survived the experimental period of 150 days, and as many as 9 of them showed liver cancer.

Table IX. Experiment 5. Effect of Muscle Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	75	130	130	80	188	+
2	♂	107	170	170	120	230	±
3	♀	145	130	150	110	551	++
4	♂	148	150	150	120	551	++
5	♀	149	110	150	100	498	+

Table X. Experiment 5. (150 Days) Effect of Muscle Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body Weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	120	130	110	454	++
2	♀	150	150	120	472	+
3	♀	140	140	110	474	++
4	♀	120	130	100	484	++
5	♀	120	150	125	496	+
6	♀	140	140	110	522	++
7	♂	180	180	160	574	+
8	♂	190	190	145	587	±
9	♀	140	150	95	588	++
10	♂	150	160	140	594	++
11	♀	130	150	115	602	++
12	♂	150	150	130	614	++
13	♂	150	170	140	635	++

−: 0(0.0%) ±: 1(7.7%) +: 3(23.0%) ++: 9(69.2%)

Effect of Brain Feeding

12 of the 40 rats, with which the experiment was started, died too early in the course of experiment. The records of all the remaining rats

are tabulated in Tables XI and XII.

Table XI. Experiment 6. Effect of Brain Feeding, based on Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	65	110	110	55	106	±
2	♂	66	150	150	95	104	±
3	♂	66	100	100	65	128	−
4	♀	68	115	115	55	56	±
5	♂	77	140	140	80	100	±
6	♂	86	105	110	70	184	+
7	♀	94	125	125	60	232	+
8	♀	97	105	110	65	170	−
9	♀	103	120	120	75	212	−
10	♀	119	120	120	55	211	±
11	♂	129	125	125	80	492	+
12	♂	134	110	110	70	330	+

Table XII. Experiment 6. (150 Days) Effect of Brain Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	115	115	105	304	++
2	♀	110	110	90	316	++
3	♂	120	120	110	324	±
4	♂	110	110	95	348	++
5	♀	100	110	105	360	+
6	♂	110	110	100	364	±
7	♂	110	110	110	364	±
8	♂	120	120	115	388	−
9	♂	130	130	100	392	±
10	♀	115	115	100	396	++
11	♀	110	110	100	412	+
12	♂	140	140	140	440	++
13	♂	140	140	130	484	++
14	♂	140	140	135	504	±
15	♂	110	150	150	524	+
16	♂	115	160	160	568	+

−: 1(6.2%) ±: 5(31.3%) +: 4(25.0%) ++: 6(37.5%)

Effect of Lung Feeding

On the 93rd day three rats died, and one of them showed cirrhotic liver. From this time on, four other rats died with more or less recognizable changes in the liver (Table XIII). 6 rats survived 150 days, when they were all killed and examined for liver changes. Table XIV shows the individual record of these rats.

Table XIII. Experiment 7. Effect of Lung Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♂	93	60	75	50	176	+
2	♂	93	100	100	100	180	−
3	♂	93	90	105	60	224	−
4	♀	96	70	80	40	164	−
5	♀	120	75	100	75	220	+
6	♂	130	60	80	60	288	+
7	♀	132	60	90	90	364	+

Table XIV. Experiment 7. (150 Days) Effect of Lungs Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	River findings
		Initial	Maximum	Final		
1	♂	85	110	100	318	++
2	♂	70	110	90	322	+
3	♂	65	115	115	322	+
4	♂	90	100	75	368	−
5	♂	55	100	85	428	++

−: 1(20.0%) ±: 0(0.0%) +: 2(40.0%) ++: 2(40.0%)

Effect of Fore Stomach Feeding

On the 99th day, one rat died with cirrhotic liver (+), and from this time on, 3 other rats died with recognizable changes in the liver (Table XV). 18 rats survived the experimental period of 150 days, and half of them showed liver cancer (Table XVI).

Table XV. Experiment 8. Effect of Fore Stomach Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	99	60	70	45	216	+
2	♀	101	65	95	90	172	±
3	♂	101	60	80	60	240	+
4	♂	138	70	75	70	308	+

Table XVI. Experiment 8. (150 Days) Effect of Fore Stomach Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♂	65	100	100	272	+
2	♀	65	100	90	284	+
3	♀	65	115	100	296	±
4	♂	70	100	100	300	−
5	♂	55	120	120	316	++
6	♀	60	90	80	320	++
7	♀	50	110	105	332	++
8	♂	60	120	115	336	−
9	♂	60	95	80	340	±
10	♂	70	130	115	348	±
11	♂	90	135	120	352	++
12	♂	60	120	120	356	±
13	♀	60	90	90	372	++
14	♀	80	110	100	372	++
15	♂	80	95	90	373	++
16	♂	75	120	120	376	++
17	♂	70	100	100	408	++
18	♂	70	140	140	426	±

−: 2(11.1%) ±: 5(27.7%) +: 2(11.1%) ++: 9(50.0%)

Effect of Glandular Stomach Feeding

As may be seen from the data given in Tables XVII and XVIII, the addition of the fore stomach did not produce any perceptible effect on the production of liver cancer. 17 rats survived 150 days, when they were killed and examined for liver changes, and 5 of them showed liver cancer.

Table XVII. Experiment 9. Effect of Glandular Stomach Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	113	85	120	120	190	−
2	♂	113	60	70	70	216	+
3	♂	129	85	95	70	268	+
4	♂	129	85	100	80	288	+
5	♂	146	140	200	165	464	+

Table XVIII. Experiment 9. (150 Days). Effect of Glandular Stomach Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	50	80	60	232	++
2	♀	65	110	110	296	+
3	♀	50	95	90	308	+
4	♂	55	95	95	312	−
5	♀	60	100	80	316	++
6	♂	60	100	90	320	++
7	♂	70	110	110	320	−
8	♀	65	115	100	328	+
9	♂	80	110	90	336	+
10	♂	90	110	100	336	+
11	♂	75	140	140	344	±
12	♂	70	120	120	344	++
13	♂	110	145	145	348	±
14	♀	85	125	125	352	++
15	♀	60	105	90	356	+
16	♂	80	130	130	360	±
17	♂	80	110	100	405	−

−: 3(17.6%) ±: 3(17.6%) +: 6(35.3%) ++: 5(29.4%)

Effect of Small Intestine Feeding

On the 137th and the 138th day, rat each died with cirrhotic liver (+) (Table XIX). As many as 18 rats survived the experimental period of 150 days, and 5 of them showed liver cancer (Table XX).

Table XIX. Experiment 10. Effect of Small Intestine Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♂	137	70	85	70	272	+
2	♀	138	60	90	65	314	+

Table XX. Experiment 10. (150 Days) Effect of Small Intestine Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	65	75	70	280	++
2	♂	60	85	70	292	+
3	♀	55	90	80	336	±
4	♂	65	95	95	340	+
5	♂	55	80	80	342	++
6	♂	65	110	100	348	±
7	♀	70	100	90	348	++
8	♂	65	100	95	356	±
9	♂	65	110	100	376	++
10	♂	65	135	120	380	−
11	♂	100	110	110	384	−
12	♂	55	100	90	388	++
13	♂	75	100	90	392	±
14	♀	84	100	90	396	+
15	♂	60	120	110	404	+
16	♂	75	120	110	408	±
17	♂	75	130	130	412	±
18	♂	100	130	110	420	−

−: 3(16.6%) ±: 6(33.3%) +: 4(22.2%) ++: 5(27.8%)

Effect of Pancreas Feeding

5 rats died before the 150 day period, and 3 of them showed cirrhosis in the livers (Table XXI). Other 5 rats survived the experimental period of 150 days, and all showed liver cancer (Table XXII).

Table XXI. Experiment 11. Effect of Pancreas Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	97	100	100	60	132	+
2	♀	106	70	80	40	204	−
3	♂	114	80	85	40	180	−
4	♂	116	85	85	35	180	+
5	♂	125	75	75	65	328	+

Table XXII. Experiment 11. Effect of Pancreas Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♂	60	100	85	336	++
2	♀	90	90	90	336	++
3	♀	75	90	90	340	++
4	♂	65	100	100	384	++
5	♂	105	120	110	408	++

++: 5(100.0%)

Effect of Testicle Feeding

In this case, the experimrnt was started with 40 rats, 15 of which died too early to be taken into account. On the 75th day, one rat died with liver showing slightly uneven surface, and from this time on, 18 other rats died with more or less cirrhotic livers, as is shown in Table XXIII.

Table XXIII. Experiment 12. Effect of Testicle Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	75	65	90	70	116	±
2	♂	87	70	80	75	156	±
3	♂	89	90	120	110	232	−
4	♂	91	80	100	60	164	−
5	♂	96	80	110	75	118	+

6	♂	98	70	90	80	176	±
7	♂	102	60	90	50	284	−
8	♀	109	60	80	60	212	−
9	♂	114	90	130	75	420	±
10	♂	114	80	90	50	260	−
11	♂	119	100	115	80	332	+
12	♀	123	60	90	50	256	±
13	♂	126	90	110	70	384	±
14	♀	130	60	80	50	308	++
15	♂	134	100	130	90	400	+
16	♀	137	70	90	60	292	+
17	♀	140	100	100	150	332	+
18	♂	146	65	100	65	400	++
19	♂	148	90	100	55	456	+

Table XXIV. Experiment 12. (150 Days) Effect of Testicle Feeding, based on the Rats killed on the 150th Day.

Rat No.	Sex	Bady weight (g)			Amount of butter yellow ingested (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	80	100	85	332	+
2	♂	100	130	90	372	++
3	♀	90	110	65	408	+
4	♂	70	105	80	444	++
5	♀	65	120	95	444	+
6	♀	80	100	100	448	+
7	♀	60	100	65	452	+
8	♀	90	110	85	472	++
9	♂	70	100	70	484	++
10	♂	100	120	110	492	++
11	♂	70	140	125	500	++
12	♂	70	145	110	516	++
13	♂	80	100	50	520	±
14	♂	130	150	115	522	+
15	♂	100	150	115	552	+
16	♂	130	150	120	664	++

−: 0(0%) ±: 1(6.2%) +: 7(43.8%) ++: 8(50.0%)

As many as 8 of the 16 rats which survived 150 days showed liver cancers (Table XXIV). The addition of the testicle has no striking effect on the production of liver cancer.

Effect of Bile Feeding

As a specific secretion product of liver cells bile cannot fail to be of interest in connection with the inhibition of liver cancer production by liver feeding. It was from this point of view that we tested the effect of bile feeding.

Whole bovine bile from 10 animals, amounting to about 1740 cc, was concentrated into approximately 500 cc in an open dish over steam bath, and 10 cc of the concentrated material was mixed with 1kg of polished rice.

Unexpectedly large number of the rats died under bile feeding and it was with some difficulty that the data presented in Table XXV were obtained. That is, as many as 31 of the 40 rats, with which the experiment was started, died too early to be taken into account in the course of the experiment.

Table XXV. Experiment 13. Effect of Bile Feeding, based on the Rats Dying before the 150 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			Amount of butter yellow ingested (mg)	Liver findings
			Initial	Maximum	Final		
1	♀	73	110	110	55	68	+
2	♂	74	155	155	70	90	−
3	♀	79	110	110	60	88	+
4	♀	79	85	85	60	117	+
5	♀	81	90	90	55	156	+
6	♀	82	185	185	135	160	+
7	♀	88	140	140	75	128	±
8	♀	89	90	90	75	168	+
9	♀	131	140	140	70	276	+

−: 1(11.1%) ±: 1(11.1%) +: 7(77.7%)

On the 73rd day, one rat died with cirrhotic liver, and the rest of animals died before the 150 day period, showing more or less recognizable liver changes. Meagre as are these data they nevertheless show that bile feeding does not inhibit the development of liver cirrhosis which may be taken to mean that liver cancer would have resulted if the animals lived longer.

Discussion and Summary

Considerable individual variation existing among rats in their susceptibility to liver cancer producing action of carcinogenic azo compounds

makes it essential that the evidence of inhibition should be based on experiments including a sufficiently large number of animals. Failure to fully realize this fact only results in piling up of data of dubious significance, without benefiting the understanding of the subject.

In this laboratory it was demonstrated that liver feeding very strikingly inhibited the liver cancer production due to dimethylaminoazobenzol. This work has now been extended, and in the present paper effect of feeding beef tissues other than liver was described, the tissues tested being kidney, spleen, skeletal muscle, brain, lung, fore and glandular stomachs, small intestine, pancreas, testicle, and also bile. In these tests, kidney feeding alone showed a definite inhibition of liver cancer production, and although the degree of this inhibition was not as high as that obtained by liver feeding, the results obtained on a large number of rats left no doubt that here we are dealing with something that is entirely outside of the limits of any possible experimental error.

For the purpose of a ready comparison a summary of the liver findings

Table XXVI. Summary of the Entire Experimental Results.

	No. of Rats	Liver Findings			
		Macroscopically normal (−)	Uneven surface (±)	Cirrhotic (+)	Liver cancer (++)
Liver**	23	23(100.0%)	0(0.0%)	0(1.0%)	0(0.0%)
Kidney (1)	17	7(41.1%)	7(41.1%)	2(11.8%)	1(5.9%)
Kidney (2)	20	17(85.0%)	2(10.0%)	1(5.0%)	0(0.0%)
Kidney (3)	24	23(95.8%)	1(4.2%)	0(0.0%)	0(0.0%)
Spleen	5	0(0.0%)	1(20.0%)	2(40.0%)	2(40.0%)
Muscle	13	0(0.0%)	1(7.7%)	3(23.0%)	9(69.2%)
Brain	16	1(6.2%)	5(31.3%)	4(25.0%)	6(37.5%)
Lung	5	1(20%)	0(0.0%)	2(40.0%)	2(40.0%)
Fore stomach	18	2(11.1%)	5(27.7%)	2(11.1%)	9(50.0%)
Glandular stomach	17	3(17.6%)	3(17.6%)	6(35.3%)	5(29.4%)
Small intestine	18	3(16.6%)	6(33.3%)	4(22.2%)	5(27.8%)
Pancreas	5	0(0.0%)	0(0.0%)	0(0.0%)	5(100.0%)
Testicle	16	0(0.0%)	1(6.2%)	7(43.8%)	8(50.0%)
Bile*	9	1(11.1%)	1(11.1%)	7(77.7%)	0(0.0%)
Control** (polished rice alone)	70	2(2.8%)	1(1.4%)	22(31.4%)	45(64.2%)

* 73~131 Day Experimental Days.
All other experiments were terminated on the 150th Day.
** Cited from the preceeding paper.

in all the groups of rats are shown in Table XXVI and in Fig. 1 in a graph form.

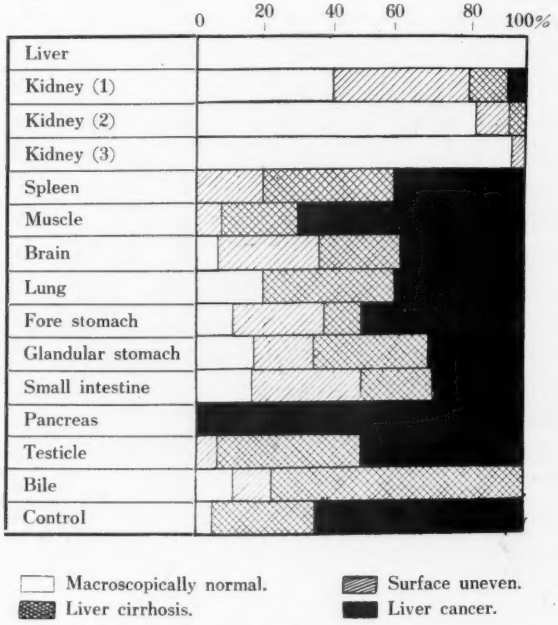

Fig. 1

The dietary influence on the experimental production of liver cancer has been made the subject of special study by *Kinosita*[8] and his associates[9], *Ando*[10-11], *Vassiliadis*[12], etc., and their results indicated the existence of liver cancer inhibiting substance in vegetable matters, namely rice bran oil and whole wheat. Our own investigations are concerned with animal tissue feeding, and here we discovered that liver and kidney exhibited a striking inhibition. Whether or not one and the same substance is responsible for the inhibiting effect of the vegetable and animal materials remains an open question, which can be settled only through further experiments.

At the end of this paper, the writer wishes to express his sincere thanks to Professor *Mataro Nagayo*, M. I. A., for his stimulating encouragement given to him and to Dr. *Waro Nakahara* for his kind guidance and valuable suggestions.

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Livers of 17 rats in Experiment 1, killed on the 150th day, showing the effect of kindney feeding on the production of liver cancer by dimethylaminoazobenzol.

Livers of 20 rats in Experiment 2, showing the effect of kidney feeding on the production of liver cancer by dimethylaminoazobenzol. All the rats were killed on the 150th day.

Liver of 24 rats in Experiment 3, showing the effect of kidney feeding on the production of liver cancer by dimethylaminoazobenzol. The rats were killid on the 150th day.

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要　旨

實驗的肝癌成生に對する臟器飼與の影響
特に腎臟飼與による抑制作用に就て

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Butter Yellow による肝癌成生實驗に關して，動物を白米に乾燥牛肝粉末を添加した飼料で飼養するに肝癌成生が顯著に抑制される事は既に確認されたところである。肝臟以外の他の臟器にもこの様な抑制作用があるであらうか。

本實驗に於ては，腎臟，脾臟，筋肉，腦髓，肺臟，前後胃，小腸，膵臟及び睾丸等の牛の諸臟器竝びに牛膽汁等を夫々10%の割合に飼料に添加して Butter yellow による肝癌成生實驗を行つた。各列40匹宛のラッテを使用し實驗開始後150日に及んで生存動物の肝臟所見を以てその影響を檢べた．尙150日以前に斃死した動物の所見も併せ比較した．

實驗の結果第XXVI表に見る如く上記の諸臟器の中唯腎臟粉のみが肝臟飼與の場合には及ばぬ程度ではあるが著しい抑制作用を示した．Azo 化合物による實驗的肝癌成生に對する食餌の抑制的影響として木下教授竝にその共同研究者は米糠油に於て，安藤或は *Vassiliadis* は小麥に就て，何づれも植物性物質を擧げてゐるが，吾々の研究に於ては動物臟器卽肝臟或は腎臟の顯著な抑制作用を證明してゐる．植物性にせよ動物性にせよ如何なる物質が抑制的效果を齎すのであらうか．實驗は進行中である將來の研究に待ちたい．

Effect of Liver Feeding on Liver Cancer Production by o-Aminoazotoluol*

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Introduction

Recent publications in this laboratory demonstrated that liver feeding very strikingly inhibited the production of liver cancer by a carcinogenic azo compound, dimethylaminoazobenzol[1,2]. In these experiments, through which this interesting fact was discovered, dimethylaminoazobenzol was administered with food, in accordance to the original method of *Kinosita*[3].

Yosida[4], *Sasaki* and *Yosida*[5] have already reported that the liver cancer can be produced with another azo compound, o-aminoazotoluol, and it has become of interest to determine whether or not the liver feeding would inhibit the production of liver cancer when o-aminoazotoluol was administered instead of dimethylaminoazobenzol.

Experiment

Two groups of 100 normal albino rats each were used in the experiment.

The first group (liver-fed) was maintained on the mixture of polished rice (900 g) and dried liver powder (100 g), supplemented with fresh carrot at the rate of a small slice per rat every other day in order not to lose the animals through too severe vitamin deficiency. The liver powder was prepared simply by drying mashed fresh beef liver, which had been sent through a meat chopper, in an open vessel over a steam bath and by pulverizing, and it was evenly mixed with polished rice. Since a small amount of olive oil (in which o-aminoazotoluol is dissolved) is added to the mix-

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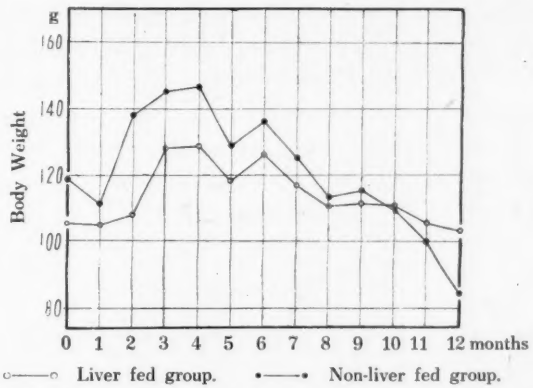
ture, the liver powder adhered fairly well to rice grains and there was little danger of rats not taking the powder with rice.

The second group (control) was fed on polished rice without liver powder, also similarly supplemented with slices of fresh carrots.

o-Aminoazotoluol was dissolved in olive oil and the solution was evenly mixed with food, and rats were allowed to feed on the mixture *ad libitum*. The amount of o-aminoazotoluol was 0.2 g per 1 kg of food (dry weight) at the beginning of the experiment, the amount being gradually increased to 1 g per 1 kg. The approximate amount of the food consumed by each rat was noted, and from it the amount of o-aminoazotoluol ingested was calculated.

Generally speaking the liver fed group appeared to remain in a slightly better physical condition than the control group, but little difference in the body weight was revealed between them as is shown in Fig. 1.

Fig. 1. Curves showing the Body Weight of Rats surviving 350 Days.



Some of the rats died early in the course of the experiment without showing noteworthy changes. The first macroscopically recognizable lesion was found in a rat in the non-liver fed group (control) dying 105 days after the commencement of the experiment, and from this time on, 59 other rats of the same group died, most of them showing more or less recognizable hepatic changes. During the corresponding period 41 rats in the liver fed group died, but in most of them the liver was free from macroscopically recognizable hepatic lesion, and only one of them showed slightly noteworthy change. And records were kept on all the rats as they died as to the liver findings (Tables I and II).

Table I. Liver Changes in the Liver Fed Group based on the Rats Dying before the 350 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			O.A.T. ingested	Liver weight (g)	Spleen weight (g)	Liver findings
			Initial	Maximum	Final				
1	♀	106	100	135	60	242	3		—
2	♂	106	80	95	60	220	4		—
3	♀	113	110	130	70	784	3		—
4	♀	113	110	110	50	216	2		—
5	♀	134	100	100	60	192	4		—
6	♀	139	90	110	100	240	2		—
7	♀	147	115	140	120	328	3		—
8	♂	147	110	110	70	284	2		—
9	♀	147	80	120	100	280	4		—
10	♂	147	90	100	55	268	2		—
11	♀	147	125	130	120	368	3		—
12	♂	148	85	120	100	340	3		—
13	♂	150	90	130	80	394	2		—
14	♀	152	100	120	70	244	3		—
15	♀	154	85	85	45	224	2		—
16	♀	168	110	120	70	352	3		—
17	♂	170	105	130	100	368	3		—
18	♀	194	120	130	95	462	3		—
19	♀	195	130	155	100	572	4		—
20	♂	196	115	130	90	540	4		—
21	♀	222	120	150	60	744	3		—
22	♂	223	120	150	85	548	9		—
23	♂	228	110	155	75	716	4		—
24	♀	250	80	100	45	520	3		—
25	♂	265	80	100	65	608	4		—
26	♀	274	105	130	65	920	5		—
27	♂	279	90	115	60	796	3		—
28	♀	279	80	120	60	764	4		—
29	♂	280	100	130	60	876	4	0.3	—
30	♀	281	95	140	65	968	4		—
31	♀	281	95	115	60	896	4		—
32	♂	291	100	120	80	824	6		—
33	♀	300	105	130	50	1180	4		—
34	♀	315	100	130	70	1348	2.5		—
35	♀	316	80	110	60	1332	3		—
36	♀	316	100	135	65	1536	3	0.3	—
37	♀	323	90	100	60	984	2	0.2	—
38	♂	323	90	125	55	1148	4	0.3	—
39	♀	323	110	125	50	1056	4	0.2	—
40	♀	325	110	140	105	1370	9	0.5	±
41	♀	334	100	100	65	1108	5	0.3	—

Table II. Liver Changes in the Non-Liver Fed Group based on the Rats Dying before the 350 Day Period.

Rat No.	Sex	Duration of survival days	Body weight (g)			O.A.T. ingested	Liver weight (g)	Spleen weight (g)	Liver findings
			Initial	Maximum	Final				
1	♀	105	180	190	150	260	8		±
2	♀	105	105	120	100	164	4		—
3	♂	105	140	140	100	304	5		—
4	♀	117	130	150	100	336	5		—
5	♂	125	100	110	70	252	3		+

6	♀	131	190	190	120	352	8		±
7	♀	153	80	100	80	320	4		−
8	♀	155	120	140	100	440	5		−
9	♀	157	135	160	80	420	4		−
10	♀	160	105	110	80	336	8		−
11	♀	175	100	120	80	346	8		±
12	♀	179	130	150	130	488	4		−
13	♀	185	210	210	130	476	10		±
14	♂	190	110	140	95	456	9		±
15	♀	199	115	180	115	626	9		±
16	♀	199	115	180	95	504	7		±
17	♀	200	90	120	95	820	8		±
18	♀	201	120	150	110	556	11		±
19	♀	201	115	150	110	626	9		±
20	♀	201	100	120	100	460	9		±
21	♀	202	130	140	115	560	13		±
22	♀	203	130	160	70	612	6		−
23	♀	204	130	140	110	572	12		±
24	♀	206	110	130	60	556	7		±
25	♀	206	130	150	75	456	5		−
26	♀	208	185	185	135	780	12		±
27	♀	208	110	140	80	908	5		±
28	♀	209	115	135	100	568	9		±
29	♀	210	190	190	100	648	9		±
30	♂	210	130	170	100	668	6		−
31	♀	215	115	140	80	656	8		±
32	♀	215	130	150	110	620	11		±
33	♂	220	130	160	85	630	10		±
34	♀	221	125	130	75	100	8		±
35	♂	224	120	160	80	772	9		±
36	♀	224	140	160	100	1264	9		±
37	♀	229	120	160	80	752	6		±
38	♀	229	115	150	100	716	10		±
39	♂	229	140	90	95	818	7		±
40	♀	239	120	170	80	776	7		−
41	♀	240	130	150	100	702	8		±
42	♂	248	100	180	65	794	5		±
43	♂	249	130	160	90	872	11		±
44	♂	252	120	140	60	884	8		−
45	♀	260	125	150	80	702	6		±
46	♂	261	150	150	90	870	12		++
47	♀	263	120	150	85	927	9		±
48	♀	270	110	150	75	1062	6		±
49	♀	271	135	160	95	1036	8		++
50	♂	271	105	150	90	1116	7		±
51	♂	276	100	130	80	892	8		±
52	♂	293	100	120	65	856	8	0.3	+
53	♂	293	115	160	80	1150	14	0.3	+
54	♀	295	150	155	110	1244	14	0.3	+
55	♀	296	160	190	110	1286	14	0.5	++
56	♂	308	120	140	75	1382	8	0.3	±
57	♀	331	125	180	70	1392	10	0.3	±
58	♀	332	110	125	60	1504	9	0.2	+
59	♂	333	120	160	100	1140	8	0.3	±
60	♂	343	110	130	60	1206	7.5	0.5	±

Experiment was discontinued at 350 days after the beginning, when all the rats then living were sacrificed for examination. At that time there were 38 rats in the liver fed and 24 in the non-liver fed (control) group. The results of the above experiment are summarized in the following tables (Tables III and IV), where the body weight (initial, maximum and final),

Table III. Liver Changes in the Liver Fed Croup, 350 Days after the Beginning of the Experiment.

Rat No.	Sex	Body weight (g)			O.A.T. ingested (mg)	Liver weight (g)	Spleen weight (g)	Liver findings
		Initial	Maximum	Final				
1	♀	90	150	95	936	8.5	0.3	−
2	♀	85	120	75	960	7	0.4	−
3	♂	95	130	95	1188	5.5	0.4	±
4	♂	90	110	105	1200	8	0.4	−
5	♀	100	170	105	1220	6.5	0.4	−
6	♂	105	135	100	1232	6.5	0.3	−
7	♀	115	130	110	1272	8	0.4	−
8	♀	100	130	110	1272	7.5	0.4	−
9	♀	100	140	90	1292	7	0.2	−
10	♀	110	150	115	1300	7	0.3	−
11	♀	80	100	95	1336	6	0.4	−
12	♂	120	150	120	1352	7.5	0.3	±
13	♀	85	120	80	1360	6	0.5	±
14	♀	75	130	125	1372	6.5	0.5	−
15	♀	110	120	85	1392	7.5	0.2	±
16	♀	125	135	120	1404	7	0.5	±
17	♂	80	120	95	1408	9	0.2	++
18	♀	90	125	105	1410	6	0.4	−
19	♂	80	100	85	1412	7.5	0.4	±
20	♀	130	150	95	1430	5.5	0.2	−
21	♀	100	125	85	1440	11.5	0.2	−
22	♀	100	145	90	1440	9	0.3	−
23	♀	100	130	100	1444	6	0.5	±
24	♂	100	135	90	1476	6.5	0.3	±
25	♂	120	135	100	1484	7	0.5	±
26	♂	100	140	85	1496	6.5	0.2	−
27	♀	95	130	125	1508	7.5	0.8	−
28	♂	120	150	110	1512	8	0.4	±
29	♂	110	130	110	1520	7.5	0.4	−
30	♀	165	165	105	1536	9.5	0.3	−
31	♀	110	150	120	1608	5.5	0.2	−
32	♂	120	150	120	1632	7.5	0.3	−
33	♀	100	150	90	1674	7.5	0.2	±
34	♀	110	150	125	1696	8.5	0.45	−
35	♂	110	135	105	1768	6.5	0.5	±
36	♀	115	170	130	1844	8	0.6	−
37	♂	80	110	90	2016	6.5	0.4	++
38	♀	100	130	120	2240	8	0.4	±

−: 23(2.8%) Macroscopically normal. ±: 13(34.2%) Hypertrophy or slightly uneven surface. +: 0 Cirrhotic. ++: 2(5.2%) Liver cancer

Table IV. Liver Changes in the Non-Liver Fed Group, 350 Days after the Beginning of the Experiment.

Rat No.	Sex	Body weight (g)			O.A.T ingested (mg)	Liver weight (g)	Spleen weight (g)	Liver findings
		Initial	Maximum	Final				
1	♀	120	120	85	1256	9.5	0.7	+
2	♂	115	160	115	1332	11	0.7	+
3	♂	110	120	65	1336	7.5	0.6	++
4	♀	190	190	110	1376	11	0.7	+
5	♀	115	160	85	1384	9	0.6	+

6	♀	110	130	100	1392	8.5	0.5	+
7	♀	100	130	105	1396	12	0.8	+
8	♂	110	120	75	1416	15.5	1	++
9	♂	150	170	105	1464	11.5	0.5	+
10	♂	110	150	105	1468	12.5	0.9	+
11	♀	115	160	110	1488	15.5	0.6	++
12	♀	115	120	85	1500	9.5	0.5	++
13	♀	120	180	75	1610	6.5	0.5	+
14	♀	120	180	115	1612	13.5	0.8	++
15	♀	115	140	85	1622	12	0.7	+
16	♀	110	150	90	1646	11	0.5	+
17	♂	110	160	120	1648	11	0.5	++
18	♀	140	180	125	1660	17.5	0.9	+
19	♀	115	185	85	1672	7.5	0.5	++
20	♀	120	120	105	1758	10.5	0.5	++
21	♀	120	170	120	1760	10.5	0.8	+
22	♀	125	140	85	1828	11.5	0.6	+
23	♀	130	150	110	1844	11	0.8	+
24	♀	125	150	110	1850	12.5	0.55	+

−: 0 Macroscopically normal. ±: 0 Hypertrophy or slightly uneven surface.
+: 16(66.6%) Cirrhotic. ++: 8(33.3%) Liver cancer.

amount of o-aminoazotoluol consumed, liver and spleen weights, and the nature of liver findings are tabulated for all the rats.

The proportions of liver and spleen weights to body weight in the case of liver fed rats was strikingly less than in the controls (Figs. 2 and 3).

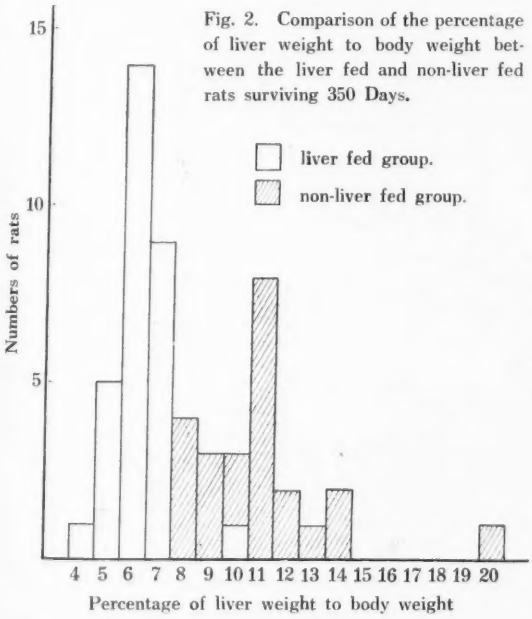


Fig. 2. Comparison of the percentage of liver weight to body weight between the liver fed and non-liver fed rats surviving 350 Days.

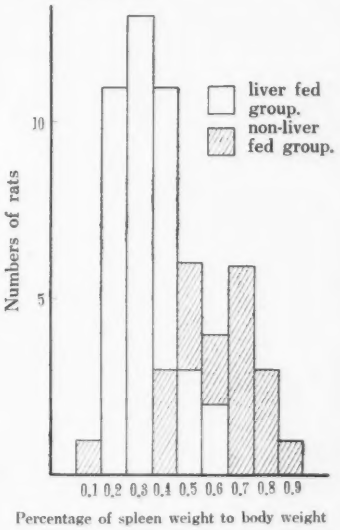


Fig. 3. Comparison of the percentage of spleen weight to body weight between liver fed and non-liver fed rats surviving 350 Days.

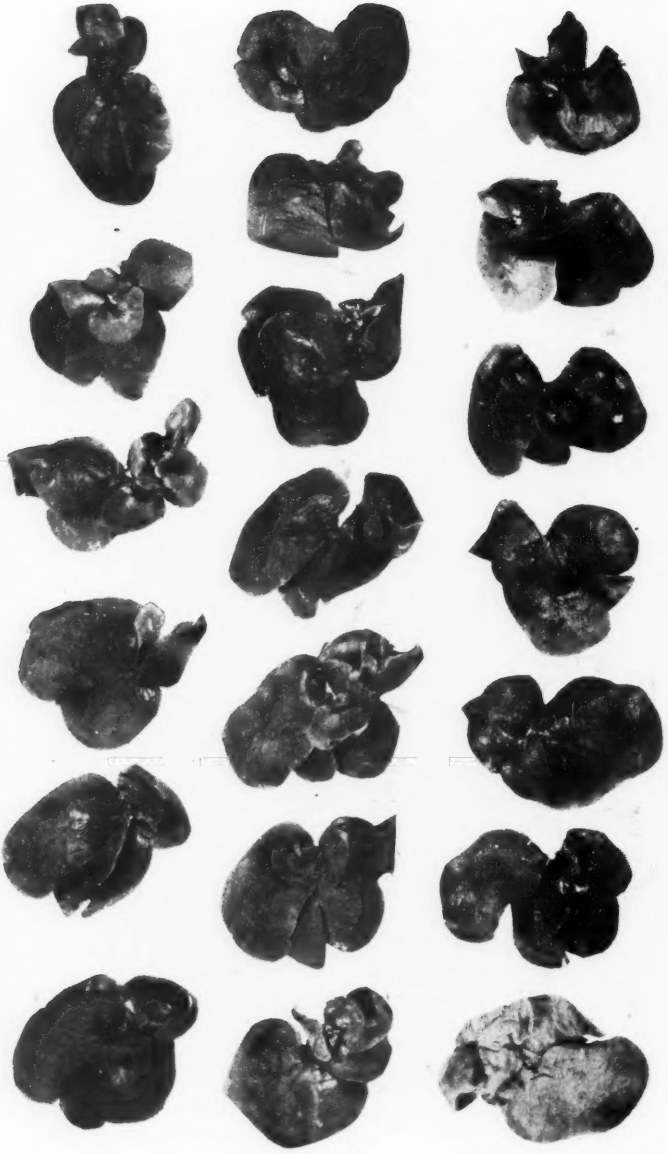
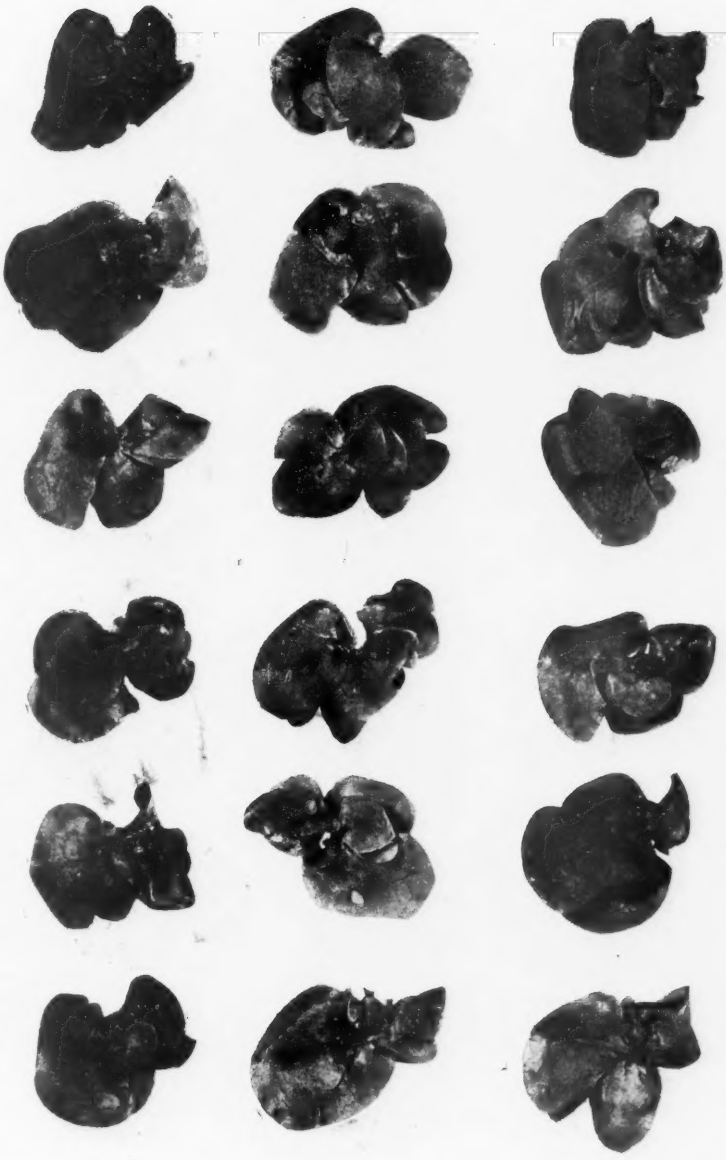


Fig. 4. Livers from the liver-fed Rats



surviving 350 Days, tabulated in Table III.

Fig. 5. Livers from the non-liver-fed Rats

surviving 350 Days, tabulated in Table IV.

In the liver fed group, 23 (2.8%) of the total 38 rats showed apparently normal liver (−), in the 13 other cases (34.2%) the liver changes were not advanced beyond the stage of somewhat granular and uneven surface (±), and only in the remaining 2 (5.2%) the hepatic changes were advanced to warrant the diagnosis of cancer, but without cirrhosis.

In the marked contrast to the conditions in the liver fed group, the non-liver fed group (control) included 8 cases (33.3%) of liver cancer accompaning cirrhosis and 16 cases (66.6%) of typical cirrhosis. The liver was macroscopically normal in none of the control rats.

The above result leaves little doubt as to the marked inhibiting effect which liver feeding exerts on the production of liver cancer by oral administration of o-aminoazotoluol.

The fact that all of the 24 non-liver fed rats surviving 350 days showed undoubted cirrhosis, including as many as 8 cases (33.3%) of liver cancer, while only 2 (5.2%) of the 38 liver fed rats, also surviving 350 days, showed liver cancer without cirrhosis, all the rest showing nothing more than slightly granular and uneven liver surface, may be regarded as striking.

Pathological Notes

Histological changes found in the variously designated livers in this experiment corresponded fairly well to those we previously described under similar designations in our paper on the inhibiting effect of liver feeding on dimethylaminoazobenzol liver cancer. Generally speaking, macroscopically normal livers (−) showed but slight histological changes, including hyperemia due to dilatation and engorgement of sinusoids, and mild proliferation of connective tissue in *Glisson*'s capsules. The enlarged livers with or without slight unevenness to their surfaces (±) contained numerous large and small vacuoles in the liver cells indicating extensive intracellular fatty deposition. These livers were also hyperemic. In the cirrhotic livers (+), the interstitial connective tissue proliferation was marked, and no small number of cases showed the picture of typical annular cirrhosis. Nodular or adenomatous hyperplasia of liver cells was common occurrence, but the proliferation of bile ducts or the formation of the so-called pseudo-bile ducts was never prominent. The liver cancer (++) encountered were all referable to hepatoma, representing various known histological types. There was no metastasis is any of them. Cholangioma was not met with in this experiment.

The histological description of liver changes leading to the production of liver cancer by o-aminoazotoluol feeding is to be found in the well known paper by *Sasaki* and *Yoshida*, and observations on our own material agree in the main with their account. We noted, however, that extensive vacuolization of liver cells and high degree of hyperemia so frequently met with in our material were not especially remarked by *Sasaki* and *Yoshida*. It is also to be emphasized that we found in our series several cases of well established cirrhosis, with the picture of typical annular cirrhosis (Fig. 6). The occurrence of cirrhosis as a forerunner of liver cancer was not recognized by *Sasaki* and *Yoshida*.

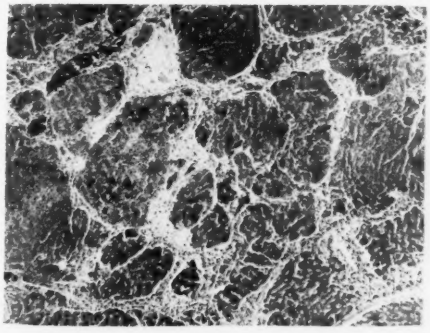


Fig. 6. Typical picture of annular cirrhosis found in o-aminoazotoluol fed rat. This form of cirrhosis has not been mentioned by previous workers on the effect of o-aminoazotoluol feeding.

As far as our observations extended, the early liver changes induced by o-aminoazotoluol seem to differ from those due to dimethylaminoazobenzol in that there is generally less marked proliferation of connective tissue in *Glisson's* capsule and hence less tendency to produce outspoken cirrhosis.

Discussion and Summary

Experimental studies on the dietary influence on the genesis of cancer has so far yielded few significant leads, and yet the subjects remains as one of the most important in the field of cancer research.

Several investigations on the acceleration or the retardation of the production of skin cancer were previously reported by such authors as *Maisin* and *Francois*[6], *Watson*[7], and *Maisin* and *Pourbaix*[8] on cancer production due to tar painting, and *Baumann* and *Rusch*[9] due to the exposure to

ultraviolet light. While, in the last few years, there appeared several publications by *Fischer-Wasels*[10], *Ando*[11,12], and *Vassiliadis*[13], all dealing with the decrease of the rate of liver cancer production by oral administration of o-aminoazotoluol. In connection with these investigations attention may also be called to the simultaneous publications of *Kinosita*[14,15], and his associates[16], on the effect of various diets on the production of liver cancer by dimethylaminoazobenzol (butter yellow), and the inhibiting effect of rice bran oil is especially noteworthy. In our laboratory it was demonstrated that liver feeding very strikingly inhibited the liver cancer production due to the administration of dimethylaminoazobenzol as mentioned above. Here the inhibition was of such an extent as to retard or to prevent the development not only of liver cancer but also of cirrhosis, the usual forerunner of liver cancer.

In the present experiment dealing with liver cancer production by o-aminoazotoluol, we found that all of the 24 non-liver fed rats surviving 350 days showed undoubted cirrhosis, including as many as 8 cases of liver cancer, while only 2 of the 38 liver fed rats, also surviving 350 days, showed liver cancer without cirrhosis and all of the remaining rats showed nothing more than slightly granular and uneven surface without the hypertrophy of the organ, demonstrating that liver feeding definitely, retarted the process of liver cancer production by this azo compocend also.

For the purpose of a ready comparison, the liver findings in these two

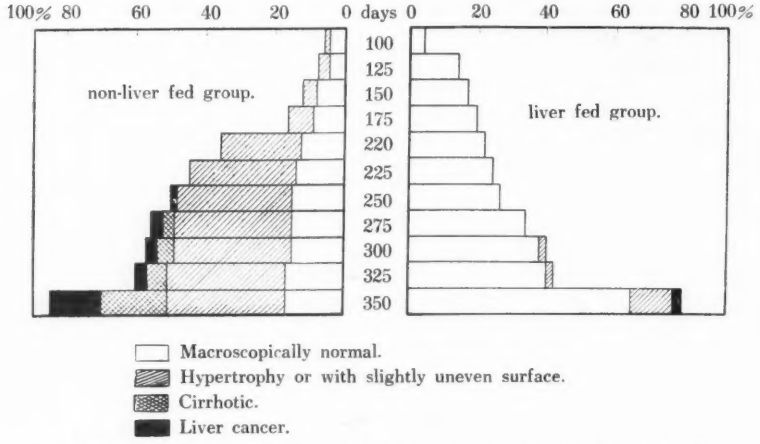


Fig. 7. Comparison of the liver findings in the rats between liver fed and non-liver fed groups, in accordance with their survival days.

groups of rats are plotted in graph form in Fig. 7, in accordance with their survival days.

In the preceding paper[17], it was demonstrated that the kidney feeding also brought about a definite inhibition of liver cancer production by dimethylaminoazobenzol, although the degree of the inhibition was not as high as that attained by liver feeding. According to the result reported in another paper[18], it is unlikely that sulphydryl compound, which occurs especially in abundance in liver and kidney, is responsible for the inhibiting action of these animal tissue feedings on the experimental liver cancer production by oral administration of dimethylaminoazobenzol or of o-aminoazotoluol. How the liver feeding brings about the inhibition of cirrhosis or hepatoma production is indeed a complex problem, and investigation is now under way in this laboratory aiming at the isolation and identification of the inhibiting substance.

Acknowledgment: The author wishes to express his gratitude to Prof. *Nagayo*, M. I. A., and Dr. *Nakahara* for their advice and encouragement during the course of the work.

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要　旨

o-Aminoazotoluol による實驗的肝癌成生に及ぼす肝臟飼與の影響

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（昭和16年1月24日受付）

Dimethylaminoazobenzol による肝癌成生實驗に際して，その飼料に牛肝粉末を添

加すると肝癌の生成が著しく抑制される事は既に報告した處である．他の Azo 化合物卽ち o-Aminoazotoluol 倒與による肝癌の生成に對しても肝粉添加は同じ樣な抑制的效果を齎らすであらうか？

各群 100 匹宛のラッテを用ひ一は白米のみを(對照群)，他は白米に 10%の割合に牛肝粉末を添加して(肝臟倒與群)倒與し，是等の動物に對して肝癌を作る樣に o-Aminoazotoluol を與へた．肉眼的に變化ある肝臟所見を示した最初の動物は，對照群に於ては實驗開始後 105 日，肝臟倒與群に於ては 325 日であつた(第 1 表及第 2 表)．

實驗日數 350 日迄生存した動物は對照群に於ては 24 匹，肝臟倒與群に於ては 38 匹であつたが。前者中 16 例 (66.6%) が肝硬變を，8 例 (33.3%) が肝癌を示したのに反して後者では 2 例 (5.2%) の肝癌を見たに過なかつた．

數年來タール塗擦による皮膚癌，或は Dimethylaminoazobenzol (Butter yellow) 又は o-Aminoazotoluol 倒與による肝癌生成に際してその遲延或は抑制作用に關する種々報告はあるが肝臟倒與のそれに勝るものはないと惟ふ．

On the Effect of Cystine Feeding on Experimental Production of Liver Cancer*

By

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(Received for Publication, January 24, 1941)

Recent publications from this laboratories reported that liver feeding markedly inhibited the production of liver cancer by carcinogenic azo compounds, dimethylaminoazobenzol[1~3] and o-aminoazotoluol[4]. And in a subsequent report[5], it was demonstrated that kidney feeding also definitely inhibited the experimental production of liver cancer by dimethylaminoazobenzol, although the degree of this inhibition was not as striking as in the case of liver feeding.

Investigation is now under way in this laboratory aiming at the isolation and identification of the inhibiting substance, and no small mass of information has already been obtained as to the nature of the substance. In the meantime, it was regarded as of interest to test the possible effect of some of the known constituents of liver and kidney, and, among these, sulphydryl compounds seemed especially worthy of consideration, not only because of their importance in cellular oxidation and reduction but also on account of their abundant occurrence in liver and kidney.

It is out of place here to go into the details as to the role of sulphydryl group in the biological oxidation and reduction. Suffice it to state that its reduced form (-SH-) takes up molecular oxygen and the resulting oxidized form (-S-S-) accepts hydrogen and re-transforms itself into the (-SH-) form. Needless to point out that such a mechanism as this offers a convenient and reasonable ground for the explanation of cellular respiration.

An additional interest in sulphydryl group is furnished by the close relation in which it stands to the phenomenon of tissue growth. This rela-

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tion has been known since the time of *Abderhalden* and *Wertheimer* and received a great emphasis from more recent workers. It is conceivable, if unlikely. therefore, that sulphydryl group, instead of suppressing the production of liver cancer, may even show the unexpected promoting effect on the carcinogenic process in question.

The present experiment was carried out with the above possibilities in mind, using l-cystine as a typical representative of the sulphydryl group. The result demonstrated, contrary to all conceptions, that the experimental production of liver cancer was neither inhibited nor promoted by cystine feeding. The details of this experiment are given in the following lines.

Experiment

Two groups of 40 albino rats each were used in this experiment.

The first group (cystine fed) was maintained on polished rice evenly mixed with l-cystine (*Takeda* Pure Chemicals, Ltd., Osaka), at the rate of 1 g per 1 kg of polished rice, which was supplemented with a small slice of fresh carrot per rat every other day so as not to allow the vitamin deficiency to become too severe for the survival of animals.

The second group (control) was kept on polished rice, also supplemented with slices of carrots exactly as in the first group.

As carcinogenic substance, dimethylaminoazobenzol (butter yellow) was used following closely the method indicated by *Kinosita*[6]. It was dissolved in olive oil, the solution was evenly mixed with basal food (polished rice with or without l cystine), and rats were allowed to feed on this mixture *ad libitum*. The amount of dimethylaminoazobenzol was 2 mg per 10 g of basal food at the beginning of the experiment, but was gradually increased, finally reaching 6 mg per 10 g. The approximate amount ingested by each rat was estimated by calculation from the total amount of the food consumed,

Some animals died early in the course of experiment, too early to show any relevant change, and they were discarded.

53 days after the beginning of the experiment one rat in the cystine fed group died, for the first time with macroscopically recognizable liver change, that is, the definitely granular and uneven surface, suggestive of early cirrhotic change (±). All the rats dying since that date were recorded as to the liver changes and in that way necessary data were secured for 16 cystine fed rats and 6 controls up to the 147th day.

As may be seen from Table I, advanced liver changes which can

Table I. Comparison of Liver Changes between Cystine Fed and Control Groups based on the Rats dying before the 150 Day Period.

Duration of survival days	1st Group — Cystin fed							2nd Group — Controls						
	Rat No.	Sex	Body weight (g)			Amount of butter yellow (mg)	Liver findings	Rat No.	Sex	Body weight (g)			Amount of butter yellow (mg)	Liver findings
			Initial	Maximum	Final					Initial	Maximum	Final		
53	1	♂	110	110	110	82	±							
58	2	♂	140	180	180	120	−	1	♀	80	90	60	90	±
60								2	♂	120	130	110	120	+
,,								3	♂	60	75	70	74	±
63	3	♀	100	170	160	88	+							
,,	4	♀	110	110	90	100	+							
74	5	♀	85	120	110	120	+	4	♀	70	90	70	112	−
,,	6	♀	115	130	130	126	+							
,,	7	♂	90	90	75	126	+							
,,	8	♀	100	110	110	135	+							
,,	9	♂	100	145	140	152	−							
,,	10	♂	120	140	125	155	±							
,,	11	♀	95	150	145	160	−							
,,	12	♀	100	150	150	166	+							
,,	13	♀	90	130	130	178	++							
81	14	♀	100	110	95	144	+							
108	15	♂	100	105	105	224	+							
111								5	♂	80	95	75	184	±
126	16	♂	130	160	150	328	++							
147								6	♂	100	110	80	294	++

definitely be diagnosed as cirrhosis (+) were found among cystine fed and control rats as early as 60~63 days after the beginning of the experiment. On the 127th day, one rat in the cystine fed group died with liver cancer, and on the 147th day liver cancer appeared also in controls.

The experiment was terminated 150 days after the beginning by sacrificing all the rats in order to make a final comparison of liver changes between the two groups, there being 16 cystine fed but only 8 control rats remaining at this time. The relevant data for these two groups of rats are tabulated in Tables II and III.

In the cystine fed group 6 (37.5%) of the total of 16 rats showed typical cirrhosis (+) and the remaining 10 (62.5%) liver cancer (++). The control group included 5 cases (62.5%) of liver cancer (++) and 3 cases (37.5%) of typical cirrhosis (+). It so happened that the percentage of liver cancer was the same in both groups.

The histological nature of the liver changes macroscopically noted and variously designated in the above tabulations as approximately normal (−),

Table II. Liver Changes in the Cystin Fed Group, 150 Days after the Beginning of the Experiment.

Rat No.	Sex	Body weight (g)			Amount of butter yellow (mg)	Liver findings
		Initial	Maximum	Final		
1	♂	60	75	70	292	+
2	♀	75	90	85	302	++
3	♂	60	85	85	310	++
4	♀	75	85	80	330	++
5	♀	70	100	100	350	+
6	♀	85	110	90	372	+
7	♀	90	100	90	381	++
8	♀	70	100	95	394	++
9	♀	80	115	110	408	++
10	♂	55	100	90	410	++
11	♂	90	115	110	420	+
12	♂	80	90	90	456	++
13	♀	85	115	110	460	++
14	♂	100	125	115	476	++
15	♂	85	120	120	477	+
16	♂	70	110	110	478	+

+: 6(37.5%) ++: 10(62.5%)

Table III. Liver Changes in the Control Group, 150 Days after the Beginning of the Experiment.

Rat No.	Sex	Body weight (g)			Amount of butter yellow (mg)	Liver findings
		Initial	Maximum	Final		
1	♀	120	130	95	328	++
2	♀	100	130	110	358	++
3	♀	105	150	125	384	++
4	♀	95	150	135	394	+
5	♀	100	150	150	434	+
6	♂	120	130	110	444	++
7	♀	130	155	145	444	++
8	♂	120	160	140	524	+

+: 3(37.5%) ++: 5(62.5%)

slightly uneven surface (±), cirrhotic (+) or liver cancer (++) need not now be gone into, as the standard and criteria adopted in our previous investigations and fully described in one of the former publications[2] were closely followed in making these designations.

Conclusions

From the results obtained in the above experiment it is evident that the feeding of sulphydryl compounds, represented by l-cystine, has no effect on the experimental production of liver cancer by administration of dimethylaminoazobenzol (butter yellow). It is unlikely that the sulphydryl compound, which occurs especially in abundance in liver and kidney, affords an explanation for the inhibiting action of these animal tissue feedings on the experimental liver cancer production,

At the end of this paper, the writer wishes to express his sincere thanks to Professor *Mataro Nagayo*, M. I. A., for his stimulating encouragement given to him and to Dr. *Waro Nakahara* for his kind guidance and valuable suggestions.

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要　旨

實驗的肝癌成生に對する Cystine 飼與の影響

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（昭和16年1月24日受付）

Dimethylaminoazobenzol (Butter yellow) 飼與による肝癌成生實驗に際して，その飼料に牛肝臟粉或は腎臟粉を添加するこ著しい抑制作用を示す。肝臟或は腎臟の如何なる成分がこの抑制作用を惹起するのであらうか。之等の臟器の分析的研究も一方法であるが，肝臟或は腎臟に多分に含まれる既知の成分，例へば細胞の酸化還元に重要なる役割をもつ一成分こしての Sulphydryl 群に關しての影響を檢べるここも興味ある事こ思ふ．一方 *Abderhalden* 及び *Wertheimer* 等に指摘せられて以來多くの研究者により强調せられてゐる如く，この SH 群は組織の生長に密接の關係を有つこ云ふ事から考へるこ抑制より寧ろ促進的作用を有するかもしれないこ云ふ問題も生じるわけである。本實驗に於ては SH 群こして l-cystine を用ひた。白米 1 kg に對して 1 g 宛 l-cystine を添加し， Butter yellow-Olive 油溶液こ平等に混和して ラッテに飼與した處，實驗日數 150 日に於けるその肝臟所見は對照群こ同程度の發癌率を示した。この事は SH 群例へば l-cytine は Butter yellow による實驗的肝癌成生に何等影響を有せぬ事を示してゐる。

The Sperm of Sea-Urchin as a Biological Test Object in Roentgen Dosimetry.

By

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(From the Radiological and Pathological Divisions of the Japanese Foundation for Cancer Research, Tokyo)

(Received for Publication, January 24, 1941)

In the previous papers[1,2] it has been shown that the time of the first cleavage of sea-urchin eggs increases with increasing dose of radiation administered either to the eggs or to the sperm prior to insemination. In the course of the experiment the idea has come to us that the sperm of sea-urchin may be an excellent biological test object in the dosimetry of radiation quantity. We have now taken up this subject and have determined the radiation quantity in the depth of a water phantom biologically and compared it with the results obtained by the ionometric method.

Main advantages in using the sperm of sea-urchin are as follows:

1. At the room temperature the first cleavage of the eggs is accomplished within two hours after insemination, even if the sperm is irradiated with a moderate dose of radiation. Therefore, troublesome care, which is often needed in the method of observing the delayed killing, is quite unnecessary.

2. At a given temperature the time of the first cleavage is determined only by the radiation quantity administered and depends neither on the duration of irradiation nor on the time between irradiation and insemination so far as they are not too long, that is, the first cleavage time is not affected by the time factor as was previously demonstrated. This is not the case for the eggs of sea-urchin and most of the biological test objects hitherto studied.

3. A single drop of dry sperm is sufficient to inseminate more than millions of eggs. The volume of test object can therefore be chosen sufficiently small without the danger of introducing the statistical fluctuation. There is thus no need of taking into consideration the secondary effect in the test object such as absorption and scattering, even if soft roentgen rays

are concerned.

4. A single male and female offer the sperm and eggs sufficient to repeat the experiment several times.

5. The sperm of sea-urchin is reasonably radiosensitive, though not highly. For example, 2000 r is sufficient to produce about 20 minutes delay in cleavage, the normal time of cleavage at the room temperature being about 80 minutes.

6. The spawning season of a single species of sea-urchin lasts only a month or two. But, fortunately, those of diffenent species are evenly distributed in the year around, so that the germ cells of sea-urchin are available almost in any season. The spawing season of four common species available in Misaki district of our country is as follows:

Strongylocentrotus pulcherrimus (Bafun-uni)	February～March
Anthocidaris crassispina (Murasaki-uni)	May ～July
Mespilia globulus (Kosidaka-uni)	August ～September
Pseudocentrotus depressus (Aka-uni)	October ～December

Experimental Methods

The experimental methods are nearly the same as in the previous studies. The sperm was collected simply by mincing testes with forceps and kept in a *Petri* dish. The eggs were washed several times with sea-water by passing through gauze and decanting and allowed to stand still until needed. For irradiating the sperm, a drop of dry sperm was sealed in a water-tight paper tube and a several tubes were simultaneously exposed to roentgen rays in free air or in a water phantom.

After irradiation, irradiated dry sperm was diluted with a large volume of sea-water and a few drops of diluted sperm suspension were added to a bottle containning more than ten thousands of normal eggs. As the time of the first cleavage (i. e., the time from the moment of insemination to the moment when fifty per cent have cleaved) is appreciably affected by the temperature of habitat, the bottles were kept in a thermostat till a few of the eggs began to cleave, the temperature of the thermostat being kept at 18 ± 0.2°C throughout the experiment. A several hundreds of eggs were then fixed in formaline solution every one minute during the cleavage and the determination of the moment when fifty per cent of the eggs have just cleaved was done later on the basis of these preserved materials. Since the most of the eggs cleaved within a few minutes, the error involved

in the determination of the cleavage time was always less than half a minute.

Because of the favourable property of the sperm mentioned above (advantage No. 2) there was no need of simultaneous insemination of all the samples irradiated in one session. We often exposed more than ten samples simultaneously at different distances from the focus of roentgen tubes. And yet there was no trouble of confusion by arranging the time of insemination of different samples properly. Only three hours were sufficient for handling several samples irradiated in one session.

Two species of sea-urchin, *Pseudocentrotus depressus* and *Strongylocentrotus pulcherrimus*, were used in the present experiment.

Results and Discussion

At first, the relation between the dose administered to the sperm and the time of the first cleavage was carefully redetermined, with a roentgen apparatus of stabilivolt-type at 5 mA and 170 KV. Several different doses up to 3500 r were administered to the sperm by exposing simultaneously the samples at different distances from the focus of roentgen tube. The results of a typical experiment made with *Strongylocentrotus pulcherrimus* are given in Table I and also plotted in Fig. 1. The time of the first cleavage of the eggs obtained from different females sometimes varied within a few minutes, so that the comparison was always done with the cleavage times relative to those of controls as is given in the tables.

Besides these a few samples were irradiated with radium gamma rays and results are plotted in Fig. 1 (open circle). The results obtained with the two types of radiation are in agreement within the experimental error.

Table I. Cleavage delay caused when various doses of roentgen rays up to 3500 r were administered to the sperm in free air.

Doses	0 (Controls)	1500 r	2450 r	3000 r	3500 r
Observed cleavage time in minute	75	87	95	96	98
	74	88	94.5	95.5	96.5
	76	87	94	95.5	97
	76	88	93	96	98
Mean	75.3±0.5	87.5±0.3	94.1±0.6	95.8±0.2	97.4±0.5
Relative to controls	100	116.2±0.8	125.0±1.1	127.3±0.8	129.4±1.0

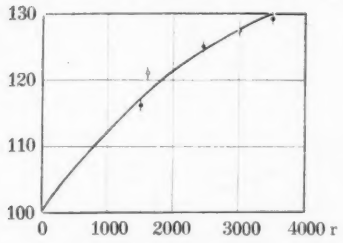

Fig. 1. Key curve showing the relation between the quantity of roentgen rays administered to the sperm and the cleavage delay caused. The ordinate represents the cleavage time relative to control and the abscissa the quantity of rays in r-unit.

The relation between the dose and the cleavage delay seems to be independent on the wave length of radiation. This fact implies the special suitability of the sperm of sea-urchin as the biological test object in the dosimetry of soft roentgen rays.

In the second experiment, the sperm was exposed to roentgen rays in the depth of a water phantom and the depth-dose was determined on the basis of the key curve given in Fig. 1 by studying the cleavage delay caused. The results obtained with the roentgen apparatus described above are given in Table II and also

Table II. Cleavage delay caused when roentgen rays of half value layer 0.87 mm Cu were administered to the sperm at various depths of a water phantom and the depth-doses estimated therefrom. The dose of incident rays at the surface of water 1470 r; Focal distance from the surface 40 cm; The field size 8×10 cm^2; Water phantom $30\times30\times25$ cm^3.

	Controls	Depth from the surface				
		0 cm	2 cm	4 cm	8 cm	12 cm
Observed cleavage time in minute	75	89	88	86	86	80
	75	89	89	87	86	80
	74	90	88	86	82.5	81
	74	90.5	88	87	83	83
Mean	74.5±0.3	89.6±0.4	88.3±0.3	86.5±0.3	84.4±0.9	81.0±0.7
Relative to control	100	120.3±0.7	118.5±0.6	116.1±0.6	113.3±1.2	108.7±1.0
Dose estimated biologically	0	2000±100 r	1750±80 r	1500±70 r	1200±120 r	750±90 r
Relative depth-dose		100	88±6	75±5	60±7	38±5
Dose determined physically	0	1970 r	1790 r	1400 r	770 r	430 r
Relative depth-dose		100	91	71	39	22

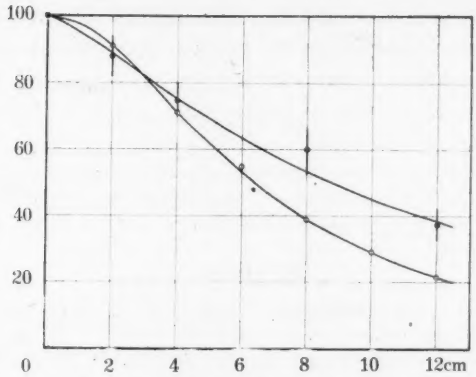

Fig. 2. Curves showing the relative depth-dose determined by biological observations and the same measured by means of an ionisation chamber (open circle). The ordinate represents relative depth-dose and the abscissa the depth from the surface of a water phantom.

plotted in Fig. 2 in conjunction with the depth-doses determined physically by using a *Matsuda* r-meter, a kind of dosimeter having a small ionisation chamber.

There is a definite discrepancy in the depth-dose between biological observations and the measurement by means of an ionisation chamber. Such a discrepancy, however, is naturally expected because the ionisation chamber used in the present experiment is more insensitive to softer roentgen rays as shown in Fig. 3 and there is much more softer rays in the depth of phantom than near the surface. Nevertheless, as the precise nature of radiation in the depth of a phantom was unknown, the variation in the spectral sensitivity of ionisation chamber was entirely neglected in the calculation of depth-dose. The discrepancy observed may be also partly due to the existence of heavier elements in the sperm which absorb the softer rays strongly.

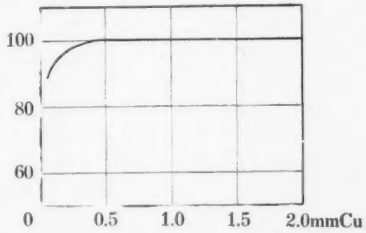

Fig. 3. Relative spectral sensitivity of *Matsuda* r-meter No. 1233. The ordinate represents the relative sensitivity and the abscissa the half value layer of roentgen rays in mm Cu.

The similar deviation but in the opposite sense was found by *Henshaw* and *Francis*[3] and also by *Packard*[4]

using different biological test objects. One can not, however, discuss much about the disagreement between the previous investigators and us because the depth-dose seems to depend not only on the nature of the ionisation chamber but also on the biological test object used.

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要　旨

海膽精蟲によるX線量の生物學的測定

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海膽精蟲にX線を當てた後通常卵を受精させるに其第一分割の時間が線量に共に增加する。本實驗に於ては此性質を逆用して水模型の種々の深さに於て精蟲にX線を當てるに伴ふ第一分割時間の遲れからX線の深部量を定めて物理的に測定せる結果と比較檢討した。其結果海膽精蟲は放射線量の生物學的測定用試驗體として從來知られてゐるものに優るとも劣らない生物なることを知つた。

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